

A MONOGRAPH OF THE VOCHYSIACEAE

IV. ERISMA¹

BY

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(Received Sept. 11th, 1954)

The genus *Erisma* is found in the Amazonian basin and in the Guianas; it comprises 16 species as defined in this paper, all of them typical Hylaeon forest trees.

The genus was first described by RUDGE in 1805; its name is a greek word meaning "a cause of dispute". RUDGE may have wanted it to refer to the romantic story of the type-specimen of his *Erisma floribundum*, a specimen belonging to a set of plants collected by the Frenchman Martin in French Guiana and captured by British privateers on its way to Paris. The name was also well suited to indicate the difficult taxonomic position of the genus.

ACKNOWLEDGMENTS

This conclusion of a revision of the *Vochysiaceae* has been made possible by the courtesy and collaboration of a great number of herbaria and institutions listed in the second part of this Monograph. The author expresses once again his deep gratitude to the directors of these institutions for the generous way in which they placed their collections at his disposal.

The revisional work has been accomplished at the Utrecht Botanical Museum and the author is greatly indebted to the director, Dr J. Lanjouw, whose stimulating interest and great help have always been of great value to him. The author is also greatly obliged to Mr N. Y. Sandwith of Kew for his constant help and assistance.

MORPHOLOGICAL REMARKS

The South-american genus *Erisma* and the Central-african *Erismadelphus* constitute the tribe *Erismeae* Dumort. of the *Vochysiaceae*. The comparative morphology of the tribe *Vochysieae* and that of the family as a whole was discussed in some detail in the second part of this Monograph. The following remarks are mainly concerned with the variation inside the *Erismeae* and its genera.

The diagram of the flower of an *Erisma* species is given in Fig. 4, a diagram of the flower of *Erismadelphus sessilis* is given by KEAY and STAFLEU (1952, p. 596).

¹ Parts I-III are listed under "References".

The calyx is very characteristic: the cup envelopes the indehiscent fruit and is crowned by the wing-shaped enlarged calyx-lobes. The spurred (fourth) lobe is deciduous in *Erisma* but persistent in *Erismadelphus*, the fruit of the former genus is therefore four-winged, that of the latter five-winged. The calyx is of the *Salvertia*-type (cf. STAFLEU 1953, p. 145): the spurred lobe is only slightly larger than the other lobes and the latter are never closely adpressed against the former.

The corolla of *Erismadelphus* consists of a complete set of five petals linking up with the quincuncial aestivation of the calyx, that of *Erisma* is reduced to a single petal corresponding with the third petal of *Erismadelphus* and *Salvertia*. Rudimentary petals are rarely present.

The only fertile stamen of *Erisma* (and *Qualea*) stands in front of the fifth calyx-lobe, outside the plane of symmetry; that of *Erismadelphus* (and *Vochysia*), on the other hand, stands in the symmetry plane at the base of the (third) petal. They obviously belong to different cycles: to an outer cycle in *Erisma*, to an inner cycle in *Erismadelphus*. The staminodes always belong to the inner cycle.

It follows that—morphologically speaking—the flowers of the African genus *Erismadelphus* are much simpler than those of the American *Erisma*: the corolla is complete and the flower is perfectly symmetrical.

The ovary of the *Erismeae* is inferior or nearly so and unilocular, that of the *Vochysieae* is superior and trilocular.

The fruits resemble the fruits of the Asian *Dipterocarpaceae* on account of the wing-shaped enlarged calyx-lobes. They contain a single wingless seed, are indehiscent and enclosed by the thickened persistent calyx-cup. The *Vochysieae* have dehiscent trilocular capsules with winged seeds.

The common feature of the inflorescence of all *Vochysiaceae* is the cincinnus. In the *Erismeae* these cincinni are arranged in ample panicles. The characters of the bracts provide the main criterion of division, between the two sections of *Erisma*. The outer bracts are always much larger than the inner ones and in the section *Erisma* they even surpass in length the flower-bud together with the distal part of the cincinnus. Young cincinni in which the first flower is still a bud are thus completely covered by the outer bract of this flower, it drops during or shortly after anthesis and then the outer bract of the next flower covers the remaining part of the cincinnus, and so on. In the section *Rixa* the outer bracts are smaller and drop earlier, the inner bracts are often absent.

GEOBOTANICAL AND ECOLOGICAL REMARKS

A peculiarity of the tribe *Erismeae* is of course its bicontinental distribution (see fig. 1). The two species of *Erismadelphus* are the only representatives of the *Vochysiaceae*, out of a total of about 190 species, outside the New World. No species are known from Atlantic or Caribbean islands.

The area of *Erisma* is strictly limited to the Amazonian basin and

the Guianas. All species but one are characteristic trees of the Hylaeian terra firme rain-forest; *Erismia calcaratum*, however, is a varzea tree and it is interesting to note that its fruits are well adapted to water

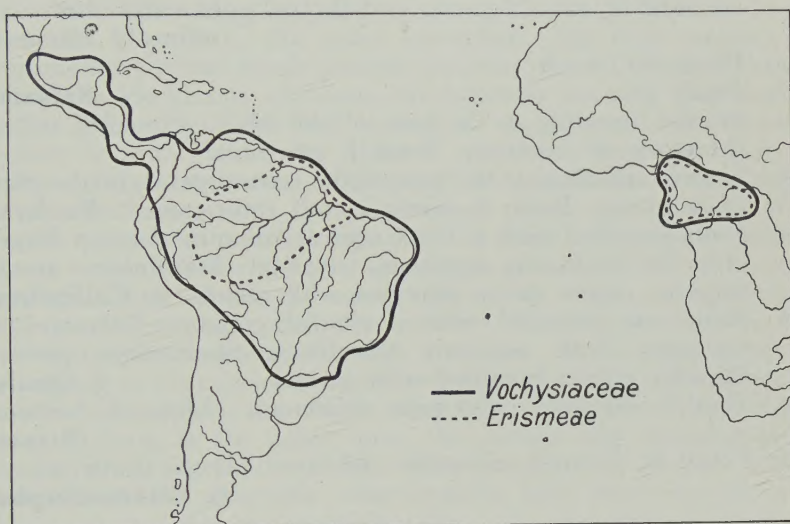


Fig. 1. Distribution of the Vochysiaceae and its tribe Erismeeae

transport. Unlike those of the other species, the wings of its fruits are poorly developed but the calyx-cup is exceptionally thick and woody.

NOTES AND ABBREVIATIONS

Most of the species of *Erismia* are of very little economic value. The wood seems to be poor but in one or two species an oil is extracted from the seeds (*E. calcaratum*, *E. japura*).

The abbreviations that have been used for the herbaria are those of the Index Herbariorum (LANJOUW and STAFLEU 1954).

Erismeeae Dumort.

Dumortier, Analyse des familles des plantes 6. 1829 ("*Erismaceae*"); Baillon 1874 p. 96, 100, 103; Chevalier & Russel 1929 p. 565; Stafleu 1952 p. 223; Keay & Stafleu 1952 p. 594.

Trees. Hairs generally stellate. Calyx semisuperior. Ovary unilocular, inferior or semi-inferior. Fruit indehiscent, enclosed by the enlarged calyx-cup and winged on account of the enlarged calyx-lobes. Seeds wingless, one per fruit.

Type genus: *Erismia* Rudge.

Distribution: Two genera, one in Amazonian South-america and one in West tropical Africa (cf. Fig. 1).

Key to the genera of the Vochysiaceae

1. a. Ovary superior, trilocular. Fruit dehiscent tribus I *Vochysieae* 2
 b. Ovary inferior or semi-inferior, unilocular. Fruit indehiscent,
 enclosed by the calyx-cup and the enlarged calyxlobes tribus II *Erismeae* 5
2. a. Petals 3, 1 or 0 3
 b. Petals 5 1. **Salvertia**
3. a. Stamen standing at the base of the fifth calyx-lobe, outside
 the plane of symmetry. Petal 1, convolute 4
 b. Stamen standing at the base of the central petal, in the plane
 of symmetry. Petals 3, rarely 1 or 0, imbricate. 2. **Vochysia**
4. a. Fruit provided with a thick central column, exocarp fragile,
 only the endocarp regularly trivalved. Staminodes absent.
 Stipular region never provided with glands. 3. **Callisthene**
 b. Fruit not provided with a central column. Exocarp and
 endocarp both regularly trivalvous. Staminodes present.
 Stipular region provided with glands 4. **Qualea**
5. a. Petal 1. Spurred calyx-lobe deciduous. (Tropical America)
 5. **Erisma**
 b. Petals 5. Spurred calyx-lobe persistent (Tropical Africa) . .
 6. **Erismadelphus**

Erisma Rudge

Rudge, Pl. Gui. 1: 7. 1805; Martius 1824 p. 136; E. Meyer 1825 p. 812; D.C. 1828 p. 29; A. Dietrich 1831 p. 101; Poeppig 1833 p. 121; Meisner 1836-43 p. 119; Endlicher 1840 p. 1179; Benth. & Hook. 1867 p. 967; Baillon 1874 p. 103; Warming 1875 p. 106; Petersen 1896 p. 319; Benoist 1915 p. 236; — 1931 p. 169; Ducke 1938 p. 38; Mennega 1948 p. 47 (wood anatomy, references); Macbride 1950 p. 872; Stafleu 1951 p. 196; Lemée 1952 p. 243. *Debraea* Roem. & Schult. 1817 p. 34, — 1822 p. 50. *Ditmaria* Spreng. 1818 p. 704, — 1825 p. 16, — 1827 p. 8. *Braya* Schult. ex Mart. 1824 p. 136 *in synonym*.

Trees. Indumentum (except where otherwise stated) consisting of stellate hairs. Perulate buds rarely present. Stipules absent or small. Leaves opposite or in whorls, simple, petioled, entire, coriaceous or subcoriaceous, penninerved, the petioles mostly canaliculate above, the midrib prominent below. Flowers hermaphrodite, irregular; cincinni 2-5 flowered, peduncled, arranged in ample panicles, accessory axes often present in the inflorescence. Pedicels bibracteate, the outer bracts larger than the inner ones. Calyx semisuperior, gamosepalous, quincuncial, densely stellate-pilose, the base cup-shaped, the limb five-parted, the lobes unequal, the fourth ("posterior", "major") lobe deciduous, spurred, irregularly suborbicular or subobcordate, two-winged, convolute, the inner wing petaloid, its inner side provided with a tuft of (mostly c. 1 mm) long simple hairs, the other lobes smaller, persistent and in nearly all species excrecent

and enveloping the fruit, the second and third ones larger than the first and fifth ones, the fifth one always subtriangular and more or less acute. Corolla, stamen and staminodes epigynously inserted on the calyx. Petal one, violaceous, yellow or white, membranous, convolute, alternating with the third and fifth calyx-lobes, caducous, generally obcordate, the apex two-lobed, the base unguiculate. Rudimentary petals rarely present. Stamen one, in front of the fifth calyx-lobe, deciduous, glabrous, the filament laterally flattened, the anther bithecate, about half as long as the filament, the base sub-hastate or emarginate, the filament attached above the base, the pollen grains triporous ("tricolpate"). Staminodes 0-4, petaloid, small, alternating with the calyx-lobes, rarely one in front of the second calyx-lobe and belonging to the cycle of the fertile stamen. Ovary nearly inferior, the free apical part pilose, unilocular. Ovules 2, epitropic, inserted on the same side as the petal, integuments 2. Style simple, not elongated after anthesis. Stigma one, capitate. Fruit indehiscent, coriaceous, ellipsoid, the base of the style persistent, remaining in the calyx-cup and crowned by the enlarged wing-shaped smaller calyx-lobes, the major wing (the third calyx-lobe) at least twice as long as the other ones, the second lobe constituting the second wing, the first and fifth lobes constituting the two smallest wings, the fifth generally subtriangular and more or less acute. Wings very small in one species but then calyx-cup strongly developed. Seeds one per fruit, oblong, not winged. Embryo homotropous, straight, radicle small, cotyledons flat.

Typification: Type species of *Erisma* Rudge (1805), *Debraea* Roem. & Schult. (1817) and *Ditmaria* Spreng. (1818, 1825): *Erisma floribundum* Rudge. The generic name *Braya* was not validly published, being given in synonymy.

Distribution: Sixteen species in the Amazonian basin and Guiana.

Ecology: Nearly all the species are found in primeval forests on terra firme.

Remarks on the subdivision: The subdivision by WARMING (1875) still holds: "Sect. I Warm." being *Sect. Erisma*, „Sect. II Warm." being named *Rixa*; the latter name is almost a linguistic synonym of the former.

Key to the species

1. a. Outer bracts dropping late, larger than the subtended flowers, enveloping the young cincinni. Inner bracts always present Section I *Erisma* 2
- b. Outer bracts deciduous, smaller than the subtended flowers, never enveloping these flowers and the distal part of the cincinnus. Inner bracts small and deciduous or absent Section II *Rixa* 11
2. a. Adult leaves pilose below 3
- b. Adult leaves glabrous or with a few scattered hairs 8
3. a. Petioles about $\frac{1}{2}$ - $1\frac{1}{2}$ cm long; blades 3-6 cm wide 4
- b. Petioles about $2\frac{1}{2}$ cm long; blades $15-25 \times 5-12$ cm 1a. **E. bicolor** Ducke var. **bicolor**
4. a. Lateral nerves strongly („costate") prominent below, leaves coriaceous 5
- b. Lateral nerves not or only slightly prominent below, leaves

- rigid 2. **E. laurifolium** Warm.
5. a. Inner bracts linear-subulate, 1–2 mm long; petioles 10–17 mm long 6
- b. Inner bracts oblanceolate-spathulate, 3–4 mm long; petioles 4–9 mm long 7
6. a. Petioles 3–3½ mm wide; leaves 4½–7 cm wide 3a. **E. costatum** Staff. var. **costatum**
- b. Petioles 1½–2 mm wide; leaves 3½–4½ cm wide 3b. **E. costatum** Staff. var. **gracilipes** Staff.
7. a. Petal glabrous 4a. **E. floribundum** Rudge var. **floribundum**
- b. Base of the petal lanuginose inside 4b. **E. floribundum** Rudge var. **tomentosum** (Ducke) Staff.
8. a. Petioles c. ½–1½ cm long; stipules present 9
- b. Petioles c. 2½ cm long; stipules absent 1b. **E. bicolor** Ducke var. **macrophyllum** (Ducke) Staff.
9. a. Leaves 6–11 × 2½–4½ cm; flower-buds 4–5 mm long 10
- b. Leaves 12–22 × 6–11 cm; flower-buds c. 10 mm long 5. **E. bracteosum** Ducke
10. a. Spur subglobose, bag-shaped, c. 1½ × 1½ mm; outer bracts c. 4 mm long 6. **E. gracile** Ducke
- b. Spur straight, slender, c. 4 mm long; outer bracts c. 7 mm long 7. **E. tessmannii** Pilger
11. a. Indumentum of the inflorescence brownish 12
- b. Indumentum of the inflorescence greyish or yellowish-greyish 16
12. a. Leaves elliptic or obovate, about twice as long as wide 13
- b. Leaves lanceolate, about three times longer than wide 8. **E. lanceolatum** Staff.
13. a. Petioles 3–10 mm long 14
- b. Petioles c. 10 mm long; lateral nerves making an angle of c. 50° with the midrib 9. **E. megalophyllum** Staff.
14. a. Spur straight, leaves glabrous below 15
- b. Spur uncinat-incurved; leaves pilose below 10. **E. fuscum** Ducke
15. a. Petioles 3–5 mm long; spur c. 1½ mm long 11. **E. micranthum** Spruce ex Warm.
- b. Petioles 8–10 mm long; spur 4–5 mm long 12. **E. nitidum** D.C.
16. a. Leaves opposite, or if verticillate spur uncinat-incurved 17
- b. Leaves verticillate; spur bag-shaped 13. **E. japura** Spruce ex Warm.
17. a. Petioles 3–6 mm long 18
- b. Petioles 10–20 mm long 19
18. a. Flower-buds up to 5 mm long; fruits winged 11. **E. micranthum** Spruce ex Warm.
- b. Flower-buds up to 10 mm long; fruits not winged 14. **E. calcaratum** (Link) Warm.
19. a. Spur uncinat-incurved; leaves pilose below

15. **E. uncinatum** Warm.
 b. Spur straight or slightly incurved; leaves glabrous
 16. **E. splendens** Staffl.

section I **Erisma**

Sect. I Warming, Fl. Bras. 13(2): 108. 1875.

Bractae persistentes vel semipersistentes, ultimae maximae axes cum floribus et bracteis insequentibus ante evolutionem tegentes.

Holotypus: *Erisma floribundum* Rudge.

1. **Erisma bicolor** Ducke, Bull. Mus. Hist. Nat. Paris II. 4: 740. 29 Nov. 1932; — 1933 p. 47, — 1935 p. 56, — 1938 p. 44. *Erisma petiolatum* Gleason, 1933 p. 363.

a. var. **bicolor**

Large tree. Young branchlets, petioles, buds, leaves below, inflorescence, bracts and calyx densely brownish pilose. Stipules absent. Leaves opposite; petioles firm, c. $2\frac{1}{2}$ cm long; leaf-blades obovate or elliptic-obovate, $15-25 \times 5-12$ cm, the apex acuminate, the base more or less obtuse or acute; lateral nerves strongly prominent below, $15-18$ on each side, angle with the midrib $50-60^\circ$, marginal nerve undulate, at $1-2\frac{1}{2}$ mm from the margin. Panicles up to 30×30 cm, many-flowered, the cincinni 2-5 flowered, up to $2\frac{1}{2}$ cm long, the peduncles up to 10 mm long, the pedicels $1-3\frac{1}{2}$ mm long, the outer bracts ovate, obtuse, up to 9×6 mm, the inner bracts subulate, c. 1 mm long, the flower-buds 6-8 mm long. Flowers fragrant. Fourth calyx-lobe violaceous, about 8 mm long and 10-12 mm wide, the spur ascending, recurved, cylindrical, c. 3 mm long. Petal white, up to 15×18 mm. Style c. 8 mm long, basal half pilose, upper half violaceous. Fruit winged, the major wing oblong, $6-8 \times 2-3$ cm, rounded, the second wing elliptic, c. 3 cm long, rounded.

Holotype: Ducke RB 23500 in RB from Borba (Brazil, Amazonas), duplicates in: G, K, NY, P, S, U, US. Holotype of *E. petiolatum* Gleason: Krukoff 1334 in NY, duplicates: A, F, G, MO, P, S, U.

Distribution: C. Amazonas and N. Matto Grosso.

BRAZIL, Amazonas: Near Borba, Rio Madeira inf., Ducke 23500; Manáos, Ducke RB 23796. Matto Grosso: Near Tabajara, upper Machado River region, Krukoff 1334 and 1376.

Ecology: On terra firme along fringes of marshes; fl. Nov.-Jan., fr. May (1 report).

b. var. **macrophyllum** (Ducke) Staffl. nov. comb. *Erisma macrophyllum* Ducke 1938 p. 44.

Small tree. Branchlets and lower surface of the leaves glabrous or nearly so and therefore leaves not "bicolor". Indumentum of the inflorescence less dense than in var. *bicolor*. Major wing of the fruit subelliptic.

Holotype: Ducke RB 34.685 in RB, along Rio Curicuriary (Brazil, Amazonas), duplicates in: K, US.

Distribution: Upper Amazonia.

BRAZIL, Amazonas: Rio Curicuriary, Ducke RB 34685; São Paulo de Olivença, Krukoff 8892.

Ecology: On terra firme, fr. Febr. (one report).

2. **Erisma laurifolium** Warm. Fl. Bras. 13(2): 109. t. 20. 1875; Petersen 1896 fig. 173 A-F; Ducke 1938 p. 42. *Qualea laurifolia* Spruce ex Warm. l.c. in synon.

Large tree. Branchlets, petioles, buds, leaves below, inflorescence, bracts and calyx densely pilose. Buds pedicelled. Stipules absent. Leaves opposite; petioles c. $1-1\frac{1}{2}$ cm long, articulate at the base; leaf-blades oblong or elliptic-oblong, $11-13 \times 4-5\frac{1}{2}$ cm, often folded, the apex abruptly long-acuminate, rarely obtuse, the base obtuse; lateral nerves slightly prominent below, 10-15 on each side, angle with the midrib 60-80°, anastomosing near the margin, marginal nerve absent. Panicles provided with 2-4(-7) flowered cincinni, the peduncles up to 10 mm, the pedicels up to 3 mm long, outer bracts ovate, obtuse, the first one $8-10 \times 5-8$ mm, inner bracts lanceolate, obtuse, the first one $3-5 \times 1-2$ mm, flower-buds 6-8 mm long. Fourth calyx-lobe up to 8×8 mm, the spur patent, (slightly) incurved, up to 4 mm long. Petal white-yellowish, c. $10-13 \times 8-10$ mm, deeply two-lobed. Style pilose on the lower $\frac{2}{3}$ part. Fruit winged, the major wing oblong, $5-6 \times 1\frac{1}{2}-2$ cm, the second wing subelliptic, rounded, $2-3 \times 1-1\frac{1}{2}$ cm.

Holotype: Spruce 2889 in C, from Vaupés River (Brazil, Amazonas), duplicates in: BR, G, GOET, K, LD, NY, OXF, P, W; photograph of the C holotype in GH and F.

Distribution: Upper Amazonia.

BRAZIL, Amazonas: Vaupés River, Spruce 2889; Rio Curicuriary, Ducke RB 34681; São Paulo de Olivença, Ducke RB 34680; Rio Solimões, Belém, Froes 23745.

Ecology: On terra firme in or along marshy places; fl. Dec.-Febr., fr. Febr. (one report). "Perhaps the loftiest tree from which I have taken specimens" Spruce in mss.

3. **Erisma costatum** Stapf, nov. spec. (fig. 2).

Sectio *Erisma*. Ab *E. floribundo* Rudge petiolis longioribus, foliis subtiliter stellato-pilosis, nervis lateralibus paucioribus supra haud impressis, bracteis interioribus subulatis minimis differt.

a. var. **costatum**

Arbor. Ramuli juveniles, nervorum laterálium et costae pagina inferior, inflorescentia, calyx extra indumento denso fulvo instructi. Stipulae nullae. Gemmae ut in *E. laurifolio* pedicellatae. Folia opposita, petiolo crasso, c. $10-14$ mm longo, $3-3\frac{1}{2}$ mm lato, lamina late elliptica, c. $7-11$ cm longa, c. $4\frac{1}{2}-7$ cm lata, apice obtuse apiculata vel breviter acuminata, basi abrupte in petiolum attenuata, nervis supra haud impressis, lateralibus subtus fortiter prominentibus, utrinque $10-12$, mediis sub angulo c. $40-60^\circ$ e costa ortis nervo limbali undulato margini proximo ($1-2$ mm) junctis. Paniculae cincinnis ad $2\frac{1}{2}$ cm longis, bracteis exterioribus late ovatis, rotundatis, ad 9 mm longis, interioribus subulatis, $1-2$ mm longis, alabastris $5-6$ mm longis instructae. Calycis lacinia quarta calcare cylindrico, recurvo, c. $4-5$ mm longo instructa. Petalum album basi flavum, ad 12×12 mm. Stamen glabrum. Staminodia c. $\frac{1}{2}$ mm longa. Stylus c. 6 mm longus, basi pilosus.

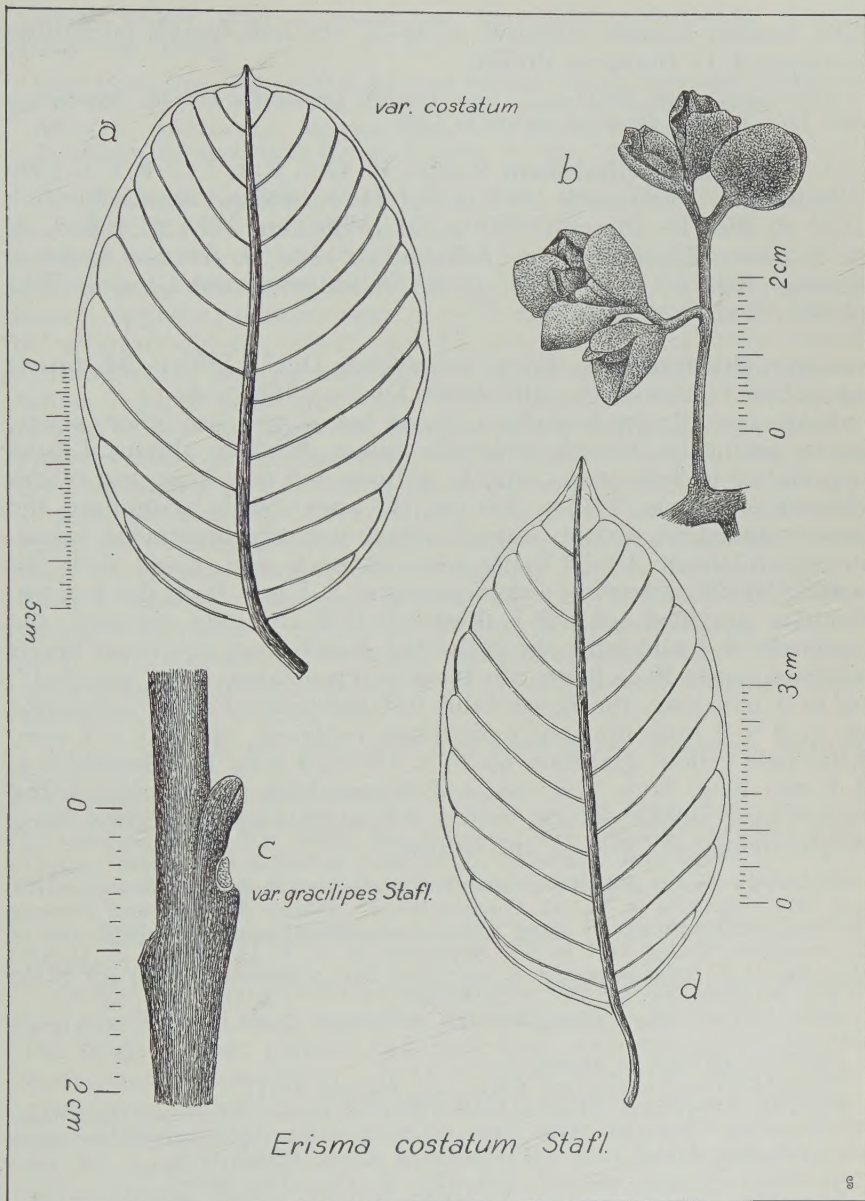


Fig. 2. *Erisma costatum* Stafl. a & b.: var. *costatum*, a. leaf, b. bracteate cincinnati; c. & d.: var. *gracilipes* Stafl., c. "pedicelled" bud, d. leaf

Holotypus: Ducke 1842 in U from Tabatinga (Goiás, Brazil) in terra firme, fl. Nov., duplicates in F, GH, NY, RB, US, (= RB 60.347).

b. var. **gracilipes** Stafl. nov. var.

A var. *costato* petiolis gracilibus, c. 13–17 mm longis, c. $1\frac{1}{2}$ –2 mm

latis, laminis anguste ellipticis, c. $3\frac{1}{2}$ – $4\frac{1}{2}$ cm latis, nervis lateralibus utrinque 9–11 instructis differt.

Holotypus: Fróes 23921 in U from Igarapé Jandiatuba (Brazil, Amazonas), defl. Jan., in terra firme, duplicate in IAN.

4. **Erisma floribundum** Rudge, Pl. Gui. Rar. 1: 7–8. *t. 1*. 1805 (“*floribunda*”); Trattinick 1811 p. 70; D.C. 1828 p. 30; A. Dietrich 1831 p. 101; D. Dietrich 1839 p. 21; Warming 1875 p. 108. *t. 21* fig. 2. *Debraea floribunda* Roem. & Schult. 1817 p. 34. *Ditmaria floribunda* Spreng. 1825 p. 16, — 1827 p. 8. *Erisma parvifolium* Gleason 1933 p. 362; Ducke 1938 p. 43.

a. var. floribundum *Erisma pallidiflorum* Ducke 1935 p. 54. *Erisma parvifolium* Gleason var. *pallidiflorum* Ducke 1938 p. 43.

Large tree. Young branchlets, leaves below, petioles, inflorescence, bracts and calyx densely brownish pilose. Stipules absent. Leaves opposite or in 3–4 merous whorls; petioles 4–9 mm long; leaf-blades obovate or elliptic, 9 – 16×3 – 6 cm, the apex shortly acuminate, the base rounded or obtuse; lateral nerves strongly prominent below, impressed above, 12–17 major ones on each side, angle with the midrib 50 – 80° , arcuately anastomosing at 2–5 mm from the margin. Panicles provided with 2–5 flowered, 2–3 cm long cincinni, the peduncles 4–7 mm long, the pedicels 1–4 mm long, the outer bracts subovate, up to 10×8 mm, the inner bracts oblanceolate, “petioled”, up to 5 mm long, the flower-buds 6–7 mm long. Fourth calyx-lobes up to 9×9 mm, the spur cylindrical, recurved, up to 5×1 mm. Petal pale yellow, glabrous, up to c. 15×15 mm. Staminodes 2–4, $\frac{1}{2}$ –1 mm long. Style glabrous, c. 5–6 mm long. Fruit winged, the major wing elliptic-oblong, c. 6×2 cm, obtuse; the second wing elliptic-oblong, c. $3 \times 1\frac{1}{2}$ cm, obtuse.

Holotype: Joseph Martin s.n. from French Guiana in BM, duplicates in BR and MO, fragments in M. (cf. remarks below). *Debraea floribunda* and *Ditmaria floribunda* are based on the same type (nomenclatural synonyms). Holotype of *E. parvifolium*: Krukoff 1401 in NY, duplicates in: A, F, G, MO, P, S, U, US. Holotype of *E. pallidiflorum* and *E. parvifolium* var. *pallidiflorum*: Ducke RB 24102 in RB, duplicates in: K, NY, P, S, U, US.

Distribution: Rare throughout the Amazonian basin. Type from French Guiana.

FRENCH GUIANA: Martin s.n.

VENEZUELA, Amazonas: Yavita, Ll. Williams 14111.

BRAZIL, Amazonas: Manáos, Ducke RB 32144; Igarapé Mioá infra Camanáos, Rio Negro sup., Ducke RB 24102. Matto Grosso: Upper Machado River region near Tabajara, Krukoff 1332, 1401; Jaturana River, Krukoff 1679.

Ecology: On terra firme, fl. Nov.–Dec., fr. Dec. (one report).

Remarks: The type specimen belonged to a set of plants collected by Joseph Martin in French Guiana and captured, on its way to France in the French warship l'Union, by two British privateers in 1803 or 1804. The plants came into the hands of Rudge who sent a set of 136 duplicate specimens to Martius (herbarium now in BR).

b. var. tomentosum (Ducke) Staf. nov. comb. *Erisma tomentosum* Ducke 1935 p. 55. *Erisma parvifolium* Gleason var. *tomentosum* Ducke 1938 p. 43.

Petal yellow with a white tip, the base unguiculate, densely lanuginose inside.

Holotype: *Ducke* RB 24100 in RB from Manáos (Brazil, Amazonas), duplicates in: K, NY, P, S, U, US. The synonyms are based on the same type.

BRAZIL, Amazonas: Manáos, *Ducke* 104, RB 24100 and 24101.

Ecology: In terra firme forests, fl. Dec., fr. Apr. (one report).

5. ***Erisma bracteosum*** Ducke, Bull. Mus. Hist. Nat. Paris II. 4: 740. 1932; Ducke 1933 p. 48, — 1935 p. 56, — 1938 p. 42.

Large tree. Branchlets and leaves glabrous. Stipules triangular, c. 3 mm long, the apex subulate, deciduous, the base slightly incrassate. Leaves opposite; petioles firm, c. 8–15 mm long; leaf-blades elliptic or subovate-oblong, 12–22 × 6–11 cm, the apex shortly obtuse-acuminate, the base rounded; major lateral nerves slightly prominent below, 7–11 on each side, angle with the midrib c. 60–70°, anastomosing at 3–7 mm from the margin, proper marginal nerve absent. Panicles when fresh white-yellowish, all parts densely fulvous pilose, the cincinni up to 4 cm long, up to 6-flowered, the peduncles up to 7 mm, the pedicels 1–2 mm long, the outer bracts suborbicular, diam. 8–11 mm, the inner bracts subulate, c. 1 mm long, the flower-buds c. 10 mm long. Flowers fragrant. Fourth calyx-lobe up to 14 × 14 mm, the spur slightly recurved, subcylindrical. Petal yellow with a small red spot, up to 25 × 25 mm. Staminodes linear, 1–1½ mm long. Style 8 mm long, the lower half pilose. Fruit winged, the major wing oblong, rounded, up to 8 × 2½ cm, the second wing suboblong, rounded, up to 4 × 1½ cm.

Holotype: *Ducke* RB 23502 in RB from Borba (Brazil, Amazonas), duplicates in: G, K, NY, P, S, U, US.

Distribution: Upper Amazonian basin.

BRAZIL, Amazonas: Borba, Rio Madeira inf., *Ducke* RB 23502; São Paulo de Olivença, *Ducke* RB 24037; São Jerônimo, Rio Solimões, *Ducke* RB 24038; Igarapé Belém, Rio Solimões, *Fröes* 23701; Camatian, *Fröes* 24014.

Ecology: On terra firme in humid forests, fl. Oct.–Jan., fr. Jan. (one report).

6. ***Erisma gracile*** Ducke, Arch. Inst. Biol. Veg. 2: 55. 1935; Ducke 1938 p. 44.

Large tree. Young branchlets subpilose. Stipules subulate, caducous, 1–3 mm long. Leaves opposite, glabrous except for a few hairs on the blade below; petioles 6–8 mm long, slender; leaf-blades subcoriaceous, ovate-elliptic, 6–11 × 2½–4½ cm, the apex obtuse-acuminate, the base subacute, the lateral nerves very slightly prominent on both sides, 6–9 major ones on each side, angle with the midrib 60–80°, anastomosing at 3–6 mm from the margin, proper marginal nerve absent. Branchlets and peduncles of the panicles moderately, the other parts densely fulvous pilose, the cincinni ½–1½ cm long, 2–4 flowered, the peduncles 2–4 mm long, the pedicels 1½–2½ mm long, the outer bracts subovate, up to 4 × 4 mm, the inner bracts linear-subulate, ½–1½ mm long, the flower-buds 4–5 mm long. Flowers when fresh white with a yellow petal. Fourth calyx-lobe c. 6 × 6 mm, the spur subglobose, pendent, diam. c. 1½ mm. Petal c. 11 × 9 mm the base pilose inside. Style 4–5 mm long, the base pilose. Fruit

winged, the major wing oblong, up to $5 \times 1\frac{1}{2}$ cm, the second wing oblong, up to 2×1 cm.

Holotype: *Ducke* RB 24103 in RB from Rio Tarumá (Brazil, Amazonas), duplicates in: K, NY, P, S, U, US.

Distribution: Collected four times from the same tree.

BRAZIL, Amazonas: Rio Tarumá near Manács, *Ducke* RB 24103, — 371.

Ecology: In rarely flooded riparian forest; fl. Dec.-Feb., fr. Apr. (one report).

7. *Erisma tessmannii* Pilger, Notizbl. Berlin 11: 295. 1931.

Medium-sized tree. Young branchlets pilose. Leaves opposite; petioles c. 5 mm long, c. 1 mm wide, canaliculate above; leaf-blades glabrous or slightly pilose in youth, elliptic, $6-8\frac{1}{2} \times 2-4$ cm, the apex shortly obtuse-acuminate, the base cuneate-rounded or rounded; the lateral nerves somewhat impressed above, slightly prominent below, 8-10 on each side, angle with the midrib 60-80°, anastomosing near the margin, proper lateral nerve absent. Panicles loose, up to 12 cm long, all parts (probably) yellowish-brown pilose, the calyx tomentose outside, the cincinni 2-3 cm long, 3-6 flowered, the peduncles c. 5 mm long, the pedicels c. 1-3 mm long, the outer bracts subovate, rounded, up to 7 mm long, the inner bracts narrow-ovate, c. 3 mm long. Fourth calyx-lobe irregularly sub-orbicular, c. 9 mm long, the spur straight, subcylindrical, narrow, up to c. 4 mm long. Petal orange-coloured. Fruit winged, the major wing oblong, rounded, c. 5 cm long and c. 1 cm wide.

Holotype: Tessmann 4932 in B (now destroyed) from Peru, R. Marañon between Iquitos and Pongo de Manseriche, San Antonio, in forest on terra firme, 135 m, fl. et fr. 14-1-1924 (photograph in F).

Remarks: No duplicate is known to the present author. Since no other material has come to hand, the above description has been based on Pilger's original description and the photograph of the holotype. The species seems to be well established, it differs from *E. gracile* by the larger flowers and the straight, narrow spur; it differs from *E. floribundum* e.g. by the small number of lateral nerves, the glabrous adult leaves, etc.

section II *Rixa* Staffl.

Sect. II Warming Fl. Bras. 13(2): 108. 1875.

Bracteeae deciduae, parvae, exteriores flores insequentes ante evolutionem haud occultantes, interiores minimae vel nullae.

Holotypus: *Erisma uncinatum* Warm.

8. *Erisma lanceolatum* Staffl. nov. spec. (Fig. 3 a & b).

Erisma verisimiliter e sectione *Rixa* Staffl. petiolis nigris, gracilibus, laminis lanceolatis apice sensim acuto-acuminatis, fructibus ala majore elongato-elliptica instructis noscendum.

Arbor magna. Ramuli vetustiores cortice in squamulas parvas fisso instructi. Stipulae nullae. Folia opposita vel ternata, petiolo gracili, nigro, glabro, c. 12-15 mm longo; lamina subcoriacea, lanceolata, lanceolato-elliptica vel oblanceolata, 10-17 cm longa, $3\frac{1}{2}-5\frac{1}{2}$ cm lata, apice sensim acuto-acuminata, basi acuta vel obtusa, supra glabra nervis haud prominentibus, subtus parcissime pilosa; nervis lateralibus utrinque 6-8, curvatis, sub angulo c. 40-50° e costa ortis, subtus

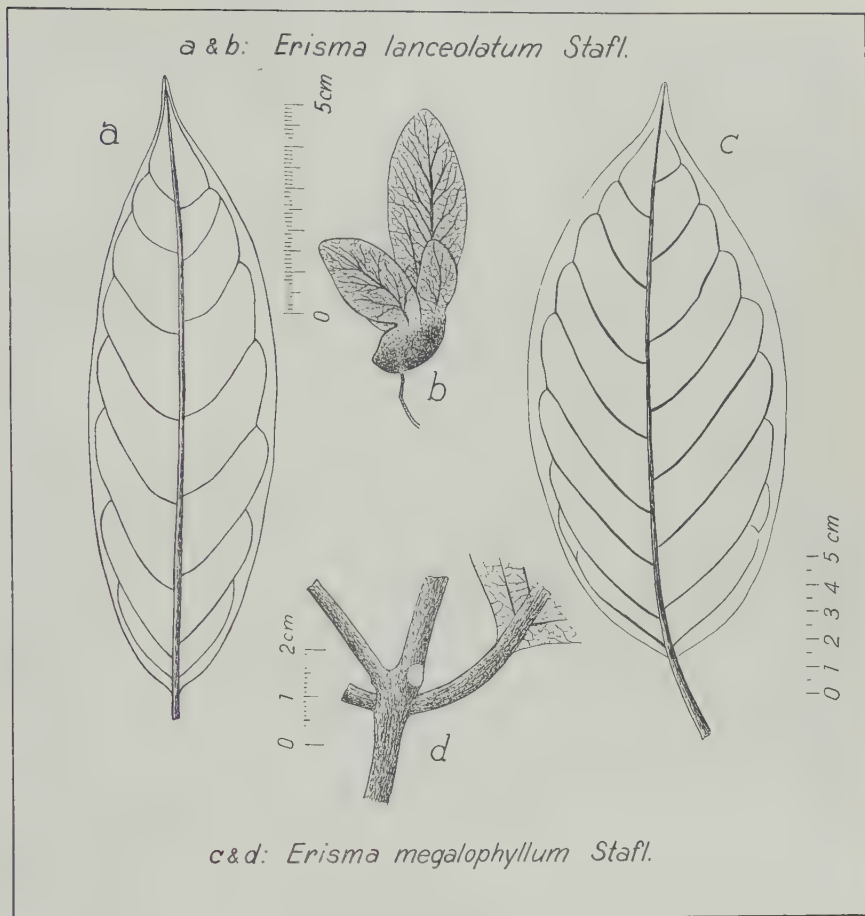


Fig. 3. a. & b.: *Erisma lanceolatum* Stafl., a. leaf, b. fruit; c. & d.: *Erisma megalophyllum* Stafl., c. leaf, d. petiole

prominentibus, prope marginem arcuatim conjunctis, neque nervo limbali junctis. Paniculae amplae, ramulis gracilibus fusco-pilosis instructae. Fructus alati, parcissime stellato-pilosi, ala majore elongato-elliptica, subrotundata, ad c. 3 cm longa et $1\frac{1}{2}$ cm lata instructi.

Holotypus: Fróes 12527/221 in A from Rio Castanha, affl. Rio Negro (Brazil, Amazonas), fl. 27-4-1942.

9. ***Erisma megalophyllum* Stafl. nov. spec.** (fig. 3 c & d).

Erisma verisimiliter e sectione *Rixa* Stafl. petiolis crassis, laminis fere glabris, magnis, apice acuto-acuminatis, nervis lateralibus utrinque 9 sub angulo c. 50° e costa ortis instructis noscendum.

Arbor media. Ramuli subpilosi demum glabrati, cortice in squamulas parvas fisso instructi. Folia ternata (irregulariter, verticem unicum vidi); petiolo crasso, c. 2 cm longo; lamina elliptica, ad 23 cm

longa et 11 cm lata, apice acuto-acuminata, basi subrotundata, supra glabra nervis haud prominentibus, subtus parcissime pilosa, nervis lateralibus utrinque 8-9, curvatis, sub angulo c. 50' e costa ortis, subtus fortiter prominentibus, prope marginem (6-9 mm) arcuatim conjunctis, neque nervo limbali junctis. Paniculae amplae, ramulis modice, pedunculis, pedicellis, calyce dense fulvo-pilosis, cincinnis ad $2\frac{1}{2}$ cm longis, 3- vel 4-floris, pedunculis c. 6-9 mm longis, pedicellis c. 1 mm longis, bracteis ignotis sed cicatricibus parvis (ergo species verisimiliter e sectione *Rixa*) instructae. Calycis laciniae prima, secunda et tertia suboblongae, obtusae, c. 3, 4-5 et 5-6 mm longae, quinta acuta, c. 4-5 mm longa, quarta decidua, ignota. Stylus c. 5 mm longus, basi pilosus.

Holotypus: *Fróes* 23741 in U, from Igarapé Belém, Rio Solimões (Brazil, Amazonas), in terra firme, defl. 18-12-1948, dupl. in IAN.

10. ***Erisma fuscum*** Ducke, Arch. Jard. Bot. Rio 4: 105. 1925; Ducke 1938 p. 44.

Large tree. Branchlets, petioles, stipules and leaf-blades below sparsely brownish pilose. Stipules sublanceolate, acute, 5-10 mm long, the apex caducous, the base persistent. Leaves opposite; petioles firm, c. $\frac{1}{2}$ cm long; leaf-blades obovate, 10-20 $\times$ 7-12 cm, the apex rounded, obtuse or apiculate, the base narrowly cordate; the lateral nerves prominent below, 9-12 on each side, angle with the midrib c. 50-60', disappearing near the margin or anastomosing, no proper marginal nerve. Panicles provided with sparsely pilose branchlets and peduncles, the other parts densely dark-brown tomentose, the cincinni up to 2 cm long, 3-5 flowered, the peduncles up to 5 mm long, the pedicels $\frac{1}{2}$ -1 mm long, the outer bracts caducous, linear, c. $\frac{1}{2}$ -1 $\frac{1}{2}$ mm long, the flower-buds 5-6 mm long. Fourth calyx-lobe c. 6 $\times$ 8 mm, the spur strongly uncinately incurved, up to 8 $\times$ 4 mm. Petal violaceous, up to 9 $\times$ 9 mm, whitish pilose on the inner side near the tip. Stamines 3-4, lanceolate, c. $\frac{1}{2}$ mm long, sometimes in serial pairs. Style c. 6 mm long, pilose at the base. Fruit winged, the major wing elongate-elliptic, up to 7 $\times$ 2 $\frac{1}{2}$ cm, the second wing elliptic, up to 1 $\frac{1}{2}$ cm long, the calyx-cup longitudinally ribbed.

Holotype: *Ducke* RB 17745 in RB from Antonio Lemos near the River Tajapurú (Brazil, Para) in terra firme forest, fl. Dec. fr. March, duplicates in G, K, P, S, U, US.

11. ***Erisma micranthum*** Spruce ex Warm. Fl. Bras. 13(2): 112. 1875.

Small tree. Branchlets and leaves glabrous. Stipules c. 1 mm long, the apex deciduous, the base incrassate and persistent. Leaves opposite; petioles 3-5 mm long; leaf-blades pergamentaceous, elongate-ovate or subelliptic, c. 9-16 $\times$ 3 $\frac{1}{2}$ -6 cm, the apex gradually acuminate or obtuse, the base more or less rounded; the lateral nerves slightly prominent, 7-11 on each side, angle with the midrib 60-80', curved upwards and anastomosing near the margin, no proper marginal nerve. Branchlets of the panicles and peduncles sparsely, the pedicels and the calyx densely greyish pilose; the cincinni up to 1 $\frac{1}{2}$ cm long,

2-5 flowered, the peduncles 2-4 mm long, the pedicels $\frac{1}{2}$ -1 mm long, the bracts caducous, the flower-buds up to 4 mm long. Fourth calyx-lobe $3-4 \times 4-4\frac{1}{2}$ mm, the spur subcylindrical, straight, c. $1\frac{1}{2}$ mm long. Petal yellow with a red base, pilose inside. Style c. 2-3 mm long, glabrous. Fruit winged, the major wing up to 10 mm long, the second wing up to 7 mm long, the calyx-cup longitudinally ribbed.

Holotype: *Spruce* 3767 in C from San Carlos, Rio Negro (Venezuela), duplicates in BR, G, GH, GOET, K, OXF, P, W).

Distribution: Upper Amazonia.

VENEZUELA, Amazonas: San Carlos, Rio Negro, *Spruce* 3767.

BRAZIL, Amazonas: Rio Iá, Rio Negro, *Fröes* 22392; Içana, Rio Negro, *Fröes* 22276; Rio Curicuriary, Rio Negro, *Ducke* RB 34.684.

Ecology: On terra firme, in high forest along river banks; fl. Nov., Dec., June (3 reports), fr. May (1 report).

12. ***Erisma nitidum*** D.C. Prodr. 3: 30. 1828; Warming 1875 p. 112; Benoist 1915 p. 237, — 1931 p. 167; Stafleu 1951 p. 199; Lemée [1952 p. 243. *Qualea lutea* Martin ex D.C. 1828 p. 30 *in synon.*

Large tree. Branchlets and leaves glabrous. Stipules c. 2-3 mm long, the apex subulate, caducous, the base partly persistent. Leaves opposite; petioles 8-10 mm long; leaf-blades thinly coriaceous, shining above, elliptic, $11-15 \times 5-7$ cm, the apex shortly obtuse-acuminate, the base subobtuse, the major lateral nerves 7-9 on each side, subprominent below, angle with the midrib c. 60-70°, the marginal nerve undulate, at 5-7 mm from the margin. Branchlets and peduncles of the inflorescence sparsely, pedicels and calyx densely fulvous pilose, the cincinni up to 3 cm long, 3-6 flowered, the peduncles up to 7 mm long, the pedicels c. 1 mm long, the outer bracts ovate, 2-3 mm long, the flower-buds 5-6 mm long. Fourth calyx-lobe $6-7 \times 7-8$ mm, the spur straight, subconical, 4-5 mm long, obtuse. Petal white with a yellow centre. Stamminodes often in serial pairs, c. $\frac{1}{2}$ mm long. Style c. 6 mm long, lower half pilose. Fruit winged, the major wing subelliptic, c. 5×2 cm, the second wing subelliptic, c. $3\frac{1}{2} \times 1\frac{1}{2}$ cm, the calyx-cup longitudinally ribbed.

Holotype: *Joseph Martin* s.n. in FI, from Roura Mt., French Guiana, duplicate in P. This specimen is also the holotype of *Qualea lutea* Martin ex D.C. *in synon.*

Distribution: French and British Guiana.

FRENCH GUIANA: Mt. Roura, *Martin* s.n.; *Leprieur* 289.

BRITISH GUIANA: Potaro River, Cobanatuk Falls, *Jenman* 7439; Potaro River, Mahdia, *For. Dept. Br. Gui.* 3743.

Ecology: In British Guiana in "Wallaba forest on white sand"; fl. Oct., Nov., fr. Nov. (one report).

Remarks: The specimens cited by *Ducke* (1933 p. 48; 1938 p. 43) as *Erisma nitidum* belong to 16 *Erisma splendens* q.v.

13. ***Erisma japura*** *Spruce* ex Warm. Fl. Bras. 13(2): 109. t. 21 fig. 1, 1875; Petersen 1896 fig. 173 G; *Ducke* 1938 p. 44.

Large tree. Branchlets, petioles, leaves below and inflorescence slightly, pedicels and calyx densely greyish pilose. Stipules small, subulate, deciduous. Leaves in tri- or tetramerous whorls; petioles $2\frac{1}{2}-4$ cm long; leaf-blades rigidly coriaceous, elliptic or obovate-elliptic, $10-23 \times 4-10$ cm, the apex rounded or nearly truncate,

emarginate, the base acute, the lateral nerves prominent below, 15–18 on each side, angle with the midrib 60–70°; the marginal nerve undulate, at 1–3 mm from the margin. Cincinni up to 5 cm long, 5–7 flowered, the peduncles 5–20 mm long, the pedicels 3–6 mm long, the outer bracts up to 10 × 5 mm, elliptic-ovate, the inner bracts absent, the flower-buds c. 12 mm long. Fourth calyx-lobe up to 14 × 18 mm, the spur bag-shaped, rounded, c. 3 × 3 mm. Petal yellow, c. 2–2½ × 2½–3½ cm. Staminodes lanceolate, c. 3½ × 1 mm. Style c. 10–12 mm long. Fruit winged, the major wing up to 13 × 4 cm, the second wing up to 7 × 2½ cm, both oblong and provided with 6–8 parallel nerves; limb ellipsoid, smooth, up to 4½ × 2½ cm, seeds oblong, c. 3 × 1 cm.

Holotype: *Spruce* 2613 in C from Rio Vaupés (Brazil, Amazonas), see remarks below. Duplicates in: BR, F, G, GH, GOET, K, NY, OXF, P, S, W.

Distribution: Northern Upper Amazonia.

BRAZIL, Amazonas: Near Panuré on Rio Vaupés, *Spruce* 2613; São Gabriel, Rio Negro, *Ducke* RB 23798; Içana, Rio Negro, *Fröes* 22290; Marabitanas, Rio Negro, *Murça-Pires* 470; Rio Curicuriary, Rio Negro, *Ducke* RB 34678.

Ecology: On terra firme; fl. Oct., Nov. April (3 reports), fr. Febr., May (2 reports).

Vernacular names: Japura (Brazil, Rio Negro).

Remarks: The flowers of *Spruce* 2613 were collected in Nov. 1852, the fruits in Febr. 1853. These collections were made (apparently) from different trees. The flowers and fruits certainly belong to the same species.

Spruce, in a mss note on the Kew specimen, says that “the kernels are pleasant eating, both raw and boiled”... “The Japurá-butter is eaten along with fish and game People who can get over its vile smell (which is never lost) find it exceedingly savoury”.

14. ***Erisma calcaratum*** (Link) Warm. Fl. Bras. 13(2): 111. 1875; Warming 1889 p. 28; Glaziou 1905 p. 31; *Ducke* 1915 p. 50, — 1922 p. 198, — 1925 p. 107 *pl.* 13 a & b, — 1938 p. 44; *Lecointe* 1934 p. 212; *Macbride* 1950 p. 873. *Qualea calcarata* Link 1820 p. 24; Roem. & Schult. 1822 p. 53. *Erisma violaceum* Mart. 1824 p. 137 t. 82; D.C. 1828 p. 30; A. Dietrich 1831 p. 102; D. Dietrich 1839 p. 21; Schomburgk 1848 p. 1099; *Baillon* 1874 p. 96 *fig.* 135, 136. *Debraea violacea* Steud. 1841 p. 486. *Ditmaria violacea* Spreng. 1827 p. 8.

Small or medium-sized tree. Branchlets and leaves glabrous. Stipules persistent, triangular, 3–5 mm long, the apex subulate, the bases widened, connate and constituting a ridge. Leaves opposite; petioles 3–6 mm long; leaf-blades generally elliptic or ovate-elliptic, 16–24 × 6–8½ cm, the apex acuminate, the base rounded or cordate; the lateral nerves subprominent below, 7–10(–12) on each side, angle with the midrib 60–80°, curved upwards, anastomosing or ending in the margin, no proper marginal nerve. Branchlets and peduncles of the inflorescence slightly, pedicels and calyx densely greyish pilose; the cincinni 3–5 mm long, 3–5 flowered, the peduncles up to 10 mm long; the pedicels 1–2 mm long, the outer bracts ovate, falling early, the inner bracts subulate, caducous, the flower-buds up to 10 mm long. Fourth calyx-lobe violaceous, c. 10 × 13 mm, the spur conical, 3–6 mm long, slightly incurved, stretched along the pedicel. Petal blue or violaceous, c. 18 × 18 mm. Staminodes

linear, c. $\frac{1}{2}$ mm long. Style glabrous, up to 9 mm long. Fruits not winged, $4-6 \times 2-3$ mm, $\pm$ oblong, slightly curved, consisting of the enlarged woody calyx-cup which almost completely encloses the true fruit, cup longitudinally ribbed and provided with numerous warts; the calyx-lobes persistent but not enlarged.

Holotype: *Hoffmannsegg* s.n. from Para (Brazil) in herbarium Willdenow in B (preserved) (the true collector is probably *F. W. Sieber*, Hoffmannsegg's servant). Since *Erismia violaceum* is an avowed synonym of *Qualea calcarata*, its holotype is also this same specimen. *Debraea violacea* and *Ditmaria violacea* are in their turn avowed synonyms of *Erismia violaceum*.

Distribution: Amazonian basin.

BRAZIL, Para: *Hoffmannsegg* (*Sieber*) s.n.; *G. A. Black* 48-2951; *Ducke* PG 10144, PG 16536, RB 17765; *Fröes* 20296; *Guedes* PG 584, PG 1665, PG 2229; *Kuhlmann* RB 17771; *Martius* s.n.; *Mexia* 6056; *Schwacke* III 556; *Sigueira* PG 3772; *Spruce* 125. Amazonas: *Manãos*, *Spruce* 1798; —, *Ducke* 897; Upper Rio Negro, *Ro. Schomburgk* 868, 898; *Padaury*, Rio Negro, *Fröes* 22677; Lower Rio Negro, *Ducke* 756; *Foz do Jatahy*, *Ducke* RB 20561.

PERU, Loreto: *Iquitos*, *Tessmann* 5330; *Mishuyaca*, *Klug* 1541.

Ecology: Typical varzea tree, fruits adapted to water transport; fl. May–Oct. from the middle of the dry season to the beginning of the “winter” fr. Nov.–Apr.

Vernacular names: *Para*: Jaboty-araconha, Jaboty, Jaboty da Varzea, Cachimbo de jaboti, Caramurú, Aburána, Vergalho de jaboti.

Use: The fruit yields an industrial oil “jaboty” used e.g. for the manufacture of soap. The wood seems to be useless as timber but can be used for the manufacture of paper.

Remarks (1) The species is mentioned from French Guiana by Lemée (1952) but this is apparently erroneous since no specimen of this species collected by *Sagot* (as indicated by Lemée) is preserved in the Paris herbarium.

(2) The fruit has the shape of a pipe and its name “Cachimbo de jaboty” is derived from this characteristic: pipe of the jaboty, a kind of terrestrial tortoise (*Ducke*).

(3) All species of *Erismia*, except the present one, are found in terra firme forests. All terra firme species have winged “dipterocarpaceous” fruits; the present species, inhabiting the regularly inundated varzea woods, has wingless fruits specially adapted to water-transport. The figure of the fruit in *Baillon* (l.c. fig. 137) does not represent the fruit of *E. calcaratum*.

15. ***Erismia uncinatum*** Warm. Fl. Bras. 13(2): 110. 1875; Wille 1882 p. 181 (anat.); Warming 1889 p. 28; Pulle 1906 p. 134; *Ducke* 1915 p. 51, — 1922 p. 198, — 1925 p. 106, — 1938 p. 44; *Benoist* 1915 p. 237, — 1919 p. 319, — 1931 p. 167, 168; *Pfeiffer* 1926 p. 357; *Lecointe* 1934 p. 211; *Amshoff* 1948 p. 11; *Mennega* 1948 p. 47 (anat.); *Macbride* 1950 p. 875; *Stafleu* 1951 p. 197; *Lemée* 1952 p. 243. *Erismia pulverulentum* Poeppig ex Warm. 1889 p. 28 (in synon.).

Large tree. Branchlets, petioles and leaves below slightly, inflorescence densely greyish (the calyx often violaceous) pilose. Stipules subulate, 1–2 mm long, the base incrassate. Leaves opposite or in tri- or tetramerous whorls; petioles 1–2 cm long; leaf-blades elongate-obovate or -oblong, $10-17 \times 4\frac{1}{2}-8$ cm, the apex rounded, obtuse or apiculate, the base cuneate, the lateral nerves 4–7(–10) on each side, angle with the midrib 40–70°, curved upwards near the margin, marginal nerve absent. Cincinni $1-3\frac{1}{2}$ cm long, 2–5 flowered, the peduncles 4–5 mm long, the pedicels c. 1 mm long, the outer bracts ovate, rounded or obtuse, 3–5 mm long, the inner bracts linear,

acute, 1–2 mm long, the flower-buds 5–7 mm long. Fourth calyx-lobe c. $5-7 \times 8-10$ mm, the spur strongly uncinately-incurved, compressed, often larger than the bud, $2-2\frac{1}{2}$ mm wide, apically rounded. Petal violaceous, $12-15 \times 12-15$ mm. Staminodes 0–4, filiform, $\frac{1}{2}-1\frac{1}{2}$ mm long, sometimes forked or in serial pairs. Style up to 7 mm long, the lower half pilose. Fruit winged, the major wings elliptic-oblong, $4-7 \times 1\frac{1}{2}-3$ and $2\frac{1}{2}-3 \times 1-1\frac{1}{2}$ cm respectively.

Typification: Warming mentions specimens collected by *Poeppig* and *Riedel*. *Poeppig* 2633 in W from Ega, Brazil, is here proposed as the lectotype. (Duplicates: BM, F, G, GOET, L, US).

Distribution: Amazonian basin and Guiana.

BRAZIL, Para: *Ducke* PG 15878, PG 16390, RB 13691; *Goeldi* PG 8322; *Guees* PG 2136; *Sigueira* PG 8827; *N.T. Silva* 136. Amazonas: *Ducke* RB 20560, RB 23797, 264 (= RB 34679); *Fróes* 23694; *Krukoff* 5603, 8084; *Poeppig* 2633; *Riedel* s.n.; *Spruce* s.n. Maranhão: Marucassumé River, *Fróes* 1939; *Assulina-Carutapera*, *Fróes* 11953.

SURINAME: *B.W.* (= *Boschwezen*) nos: 491, 1350, 1402, 1428, 2475, 3342, 3380, 3544, 3580, 4751, 5434, 5564; *Boschbeheer* 46; *Woodherbarium* 46. (Full details in Stafleu 1951 p. 198).

FRENCH GUIANA: *Benoist* 366, 1233, 1535; *Mélinon* s.n. div. coll.; *Gandoger* 8; *Wachenheim* 407.

BRITISH GUIANA: Kanuku Mts, *For. Dept. Br. G.* 5801, 5929.

Ecology: In primary forests on terra firme; fl. July–Nov., fr. Sept., Jan. (2 reports).

Vernacular names: BRAZIL, Para: Quaruba, Quaruba de flores roxas (Lecointe), Jaboty da terra firme. Maranhão: Bruto. BRITISH GUIANA: Pramaye. SURINAME: e.g. Singri-kwarrie (vide Stafleu 1951 p. 198).

16. ***Erisma splendens*** Staffl. nov. spec. (fig. 4).

Sectio *Rixa* Staffl. Ab *E. uncinato* Warm. foliis glabris, nervis lateralibus pluribus haud prominentibus, floribus indumento sordide flavescento, calcaribus subconico parum incurvo instructis, petalo intus albo-lanuginoso differt.

Arbor magna. Ramuli subpilosi demum glabrescentes, decorticantes. Stipulae 2–3 mm longae, basi incrassatae, apice subulato deciduo instructae. Folia glabra, opposita; petiolo 15–20 mm longo; lamina elliptica, 9–16 cm longa, 4–7½ cm lata, apice valde acuminata, basi cuneata, supra nitida; nervis lateralibus haud prominentibus, majoribus utrinque 9–15, sub angulo c. 60–70° e costa ortis, nervo limbali undulato margini proximo junctis. Ramuli paniculae, pedunculi parce pilosi, bracteae, pedicelli, calyx dense sordide flavescenti-pilosi. Cincinni ad 15 mm longi, 3–5 flori, bracteis exterioribus subellipticis, c. 2 mm longis, interioribus lineari-subulatis, c. ½–1 mm longis, pedunculis ad 5 mm, pedicellis ad 2 mm, alabastris c. 4 mm longis instructi. Calycis lacinia quarta c. 6 mm longa et 7 mm lata, calcaribus dependente, subconico, c. 2–2½ mm longo et 1½–2 mm lato parum incurvo instructa. Petalum extra album vel pallide lilacinum, intus flavum, dense albo-lanuginosum, c. 10–12 mm longum et latum. Petalum rudimentarium nonnunquam adest, spatulatum, dense lanuginosum, inter sep. 4 et 2 positum. Staminodia c. ½–1 mm longa. Stylus basi pilosus. Ala major fructuum elongato-elliptica, ad c. 7 cm longa et 2½ cm lata, ala secunda oblonga, ad c. 3½ cm longa et c. 18 mm lata.

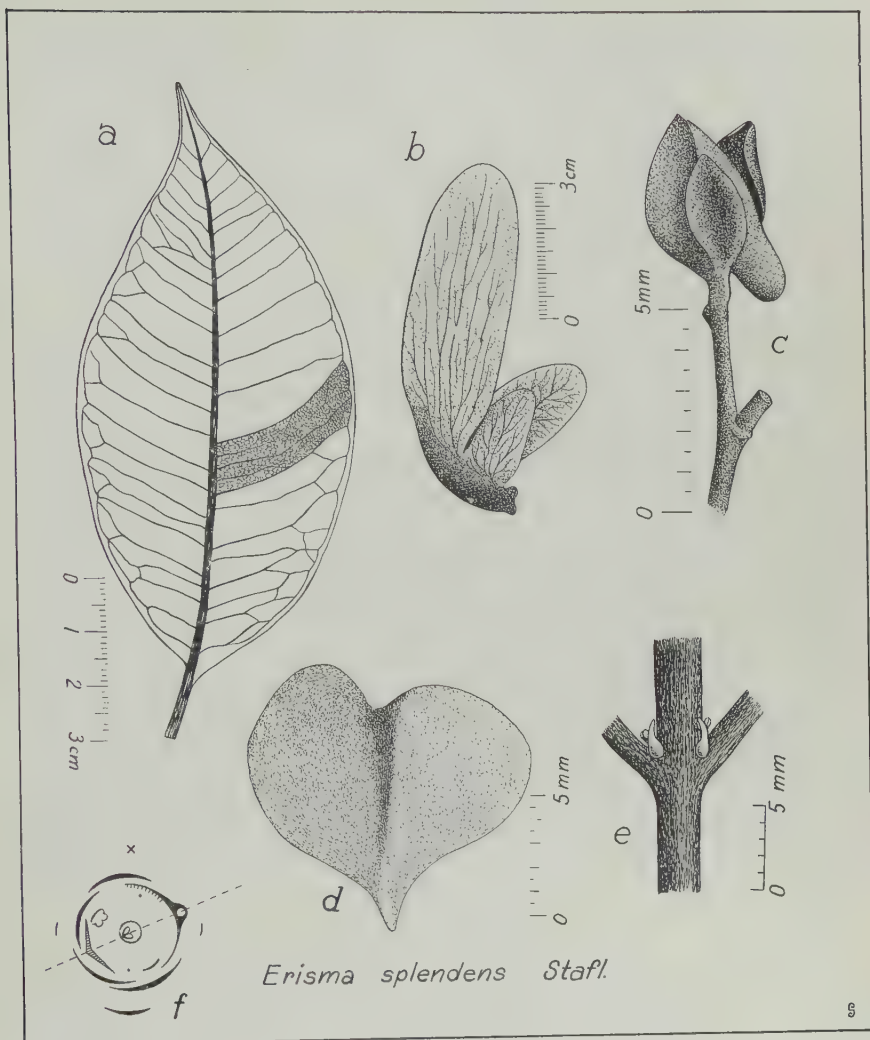


Fig. 4. *Erisma splendens* Stapf. a. leaf, b. fruit, c. flower-bud, d. petal, e. stipules, f. diagram

Holotypus: *Ducke* RB 34682 in U from Manáos (Brazil, Amazonas), duplicates in: G, K, P, S, U, US.

Distribution: Central Amazonia.

BRAZIL, Amazonas: Manáos, *Ducke* RB 34682, RB 23501, RB 34683, 96.

Ecology: In marshy forests on terra firme; fl. Nov.–Dec., fr. Feb.

Species dubiae

17. ***Erisma maliforme*** Link ex A. Dietrich, Sp. Pl. ed. 6. 102. 1831 (“*maliformis*”); Warming 1875 p. 114.

The description of this species does not enable us to run it down to one of the known species of *Erisma*. It is so incomplete that it is even uncertain whether *Link's* specimen was indeed an *Erisma*. The specimen was preserved in *Link's* herbarium and was destroyed with the other Berlin collections.

18. ***Erisma niveum*** Link ex A. Dietrich, Sp. Pl. ed. 6. 102. 1831 ("nivea"); Warming 1875 p. 114.

It is not possible to identify this species: the description is too incomplete and *Link's* original specimen was destroyed together with the Berlin collections.

Collectors' numbers

The numbers in parentheses refer to the serial numbers of the species of *Erisma*. The letter *a* refers to the subdivision containing the nomenclatural type of the species, the letter *b* to the second subdivision of that species.

BENOIST 366 (15), 1233 (15), 1535 (15). BLACK, G. A. 48-2951 (14). BOSCHBEHEER 46 (15). B.W. 491, 1350, 1402, 1428, 2475, 3342, 3380, 3544, 3580, 4751, 5434, 5564 (all 15). DUCKE 96 (16), 104 (4*b*), 264 (15), 371 (6), 756 (14), 897 (14), 1842 (3*a*), PG 10144 (14), PG 15878 (15), PG 16390 (15), PG 16536 (14), RB 13691 (15), RB 17745 (10), RB 17765 (14), RB 20560 (15), RB 20561 (14), RB 23500 (1*a*), RB 23501 (16), RB 23502 (5), RB 23796 (1*a*), RB 23797 (15), RB 23798 (13), RB 24037 (5), RB 24038 (5), RB 24100 (4*b*), RB 24101 (4*b*), RB 24102 (4*a*), RB 24103 (6), RB 32144 (4*a*), RB 34678 (13), RB 34679 (15), RB 34680 (2), RB 34681 (2), RB 34682 (16), RB 34683 (16), RB 34684 (11), RB 34685 (1*b*), RB 60347 (3*a*). FOREST DEPT. BR. GUI. 3743 (12), 5801 (15), 5929 (15). FRÓES 1939 (15), 11953 (15), 12527/221 (8), 20296 (14), 22276 (11), 22290 (13), 22392 (11), 22677 (14), 23694 (15), 23701 (5), 23741 (9), 23745 (2), 23921 (3*b*), 24014 (5). GANDOGER 8 (15). GOELDI PG 8322 (15). GUEDES PG 584 (14), PG 1665 (14), PG 2136 (15), PG 2229 (14). JENMAN 7439 (12). KLUG 1541 (14). KRUKOFF 1332 (4*a*), 1334 (1*a*), 1376 (1*a*), 1401 (4*a*), 1679 (4*a*), 5603 (15), 8084 (15), 8892 (1*b*). KUHLMANN RB 17771 (14). LEPRIEUR 289 (12). MEXIA 6056 (14). MURÇA PIRES 470 (13). POEPPIG 2633 (15). SCHOMBURGK 868 or 898 (14). SCHWACKE III 556 (14). SIGUEIRA PG 3772 (14), PG 8827 (15). SILVA, N.T. 136 (15). SPRUCE 125 (14), 1798 (14), 2613 (13), 2889 (2), 3767 (11). TESSMANN 4932 (7), 5330 (14). WACHENHEIM 407 (15). WILLIAMS, LL. 14111 (4*a*). WOODHERBARIUM 46 (15).

Vernacular names

The numbers refer to the serial numbers of the species of *Erisma*.

Aburána	14	Jaboty da varzea	14
Bruto	15	Japura	13
Cachimbo de jaboti	14	Quaruba	15
Caramurú	14	Quaruba de floras roxas	15
Jaboty or Jaboti	14	Prumaye	15
Jaboty-aracanha	14	Singri-kwarrie	15
Jaboty da terra firme	15	Vergalho de jaboti	14

Specific and infraspecific names

The numbers refer to the serial numbers of *Erisma* under which the names are cited. Names printed in *italics* are synonyms; those printed in **bold face** type refer to new taxa.

<i>Debraea</i>		<i>Ditmaria</i>	
<i>floribunda</i> Roem & Schult.	4	<i>floribunda</i> Spreng.	4
<i>violacea</i> Steud.	14	<i>violacea</i> Spreng.	14

Erisma

bicolor Ducke	1	megalophyllum Stafl.	9
var. bicolor	1a	micranthum Spruce ex Warm.	11
var. macrophyllum (Ducke) Stafl.	1b	nitidum D.C.	12
bracteosum Ducke	5	niveum Link ex A. Dietrich	18
calcaratum (Link) Warm.	14	<i>pallidiflorum</i> Ducke	4a
costatum Stafl.	3	<i>parvifolium</i> Gleason	4
var. costatum	3a	var. <i>pallidiflorum</i> Ducke	4a
var. gracilipes Stafl.	3b	var. <i>tomentosum</i> Ducke	4b
floribundum Rudge	4	<i>petiolatum</i> Gleason	1
var. floribundum	4a	<i>pulverulentum</i> Poepp. ex Warm.	15
var. tomentosum (Ducke) Stafl.	4b	splendens Stafl.	16
fuscum Ducke	10	tessmannii Pilger	7
gracile Ducke	6	<i>tomentosum</i> Ducke	4b
japura Spruce ex Warm.	13	uncinatum Warm.	15
lanceolatum Stafl.	8	<i>violaceum</i> Mart.	14
laurifolium Warm.	2		
<i>macrophyllum</i> Ducke	1b		
maliforme Link ex. A. Dietrich	17		

Qualea

<i>calcarata</i> Link.	14
<i>laurifolia</i> Spruce ex Warm.	2
<i>lutea</i> Martin ex D.C.	12

REFERENCES

- AMSHOFF, G. J. H. 1948. Enumeration of the herbarium specimens of a Suriname wood collection made by Prof. G. Stahel.
- BAILLON, J. B. C. F. 1874. Histoire des Plantes 5.
- BENTHAM, G. & J. D. HOOKER 1862-67. Genera Plantarum 1.
- BENOIST, R. 1915. Bull. Soc. Bot. France 62.
- BENOIST, R. 1919. Bull. Soc. Bot. France 66.
- BENOIST, R. 1931. Arch. Botanique 5, mém. 1.
- CANDOLLE, A. P. DE 1828. Prodrômus systematis naturalis regni vegetabilis 3.
- CHEVALIER, A. & RUSSEL 1929. Compt. Rend. Acad. Sci. 188.
- DIETRICH, A. 1831. Caroli a Linné Species Plantarum ed. 6. 1.
- DIETRICH, D. 1839. Synopsis Plantarum 1.
- DUCKE, A. 1915. Arch. Jard. Bot. Rio 1.
- DUCKE, A. 1922. Arch. Jard. Bot. Rio 3.
- DUCKE, A. 1925. Arch. Jard. Bot. Rio 4.
- DUCKE, A. 1932. Bull. Mus. Hist. Nat. Paris II. 4.
- DUCKE, A. 1933. Arch. Jard. Bot. Rio 6.
- DUCKE, A. 1935. Arch. Inst. Biol. Veg. 2.
- DUCKE, A. 1938. Arch. Inst. Biol. Veg. 4.
- DUMORTIER, B. C. 1829. Analyse des Familles des Plantes.
- ENDLICHER, S. 1836-40. Genera Plantarum.
- GLAZIOU, A. F. M. 1905. Bull. Soc. Bot. France 52, mém. 3.
- GLEASON, H. A. 1933. Bull. Torrey Bot. Cl. 60.
- KEAY, R. W. J. & F. A. STAFLEU 1952. Acta Bot. Neerl. 1 (4); also in Med. Bot. Mus. Utrecht 114.
- LANJOUW, J. & F. A. STAFLEU 1954. Index Herbariorum, part. I: The Herbaria of the World, second ed., Regnum Vegetabile 2 (1).
- LECOINTE, P. 1934. A Amazonia Brasileira 3.
- LEMÉE, A. 1952. Flore de la Guyane Française 2.
- LINK, H. 1822. Jahrb. Gewächskunde 1 (3).
- MACBRIDE, J. F. 1950. Fieldiana Botany 13 (3).
- MARTIUS, C. F. P. VON & J. G. ZUCCHARINI 1824. Nova Genera et Species Plantarum 1.
- MEISNER, K. F. 1836-43. Plantarum vascularium genera 1.
- MENNEGA, A. M. W. 1948. Suriname Timbers 1.
- MEYER, E. 1825. Nov. Act. Leop. 12.
- PETERSEN, O. G. 1896. Vochysiaceae in Engler & Prantl, Nat. Pfl. fam. 3 (4).
- PILGER, R. 1931. Notizbl. Berlin 11.

- PFEIFFER, J. Ph. 1926. De Houtsoorten van Suriname I. Med. Kol. Inst. 1926.
- POEPPIG, E. F. 1833. Frorieps Notizen 35 (8).
- PULLE, A. A. 1906. An enumeration of the vascular plants known from Surinam.
- ROEMER, J. J. & J. A. SCHULTES. 1817. Caroli a Linné Systema Vegetabilium 1.
- ROEMER, J. J. & J. A. SCHULTES 1822. Mantissa 1.
- RUDGE, E. 1805. Plantae Guianenses 1.
- SCHOMBURGK, R. I. 1848. Reisen in British Guiana 3.
- SPRENGEL, C. (K.) 1818. Anleitung etc. 2 (2).
- SPRENGEL, C. (K.) 1825. Syst. Veg. ed. 16. 1.
- SPRENGEL, C. (K.) 1827. Idem 4, Cur. Post.
- STAFLEU, F. A. 1948. A Monograph I, Rec. Trav. Bot. Néerl. 41; also in Med. Bot. Mus. Utrecht 95.
- STAFLEU, F. A. 1951. Vochysiaceae in Pulle, Flora of Suriname 3 (2).
- STAFLEU, F. A. 1952. A Monograph II, Acta Bot. Néerl. 1, also in Med. Bot. Mus. Utrecht 108.
- STAFLEU, F. A. 1953. A Monograph III, Acta Bot. Neerl. 2, also in Med. Bot. Mus. Utrecht 116.
- STAFLEU, F. A. cf. KEAY, R. W. J. & F. A. STAFLEU, 1952 and LANJOUW, J. & F. A. STAFLEU 1954.
- STEUDEL, E. G. 1841. Nomenclator Botanicus, ed. 1.
- TRATTINICK, L. 1811. Observationes botanicae 3.
- WARMING, E. 1875. Flora Brasiliensis 13 (2).
- WARMING, E. 1889. Vid. Med. Nat. För. 1889.
- WILLE, N. 1882. Overs. Kgl. Dansk. Vid. Selsk. För. 1882.

A CONTRIBUTION TO THE FERN FLORA OF
FRENCH GUIANA

BY

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(Received Sept. 3, 1954)

In recent times very little has been published on the fern flora of French Guiana. In 1918, R. BONAPARTE published a list of fern specimens, collected chiefly by Leprieur and Mélinon (p. 365: Guyane française, plusieurs collecteurs, herbier du Prince Bonaparte; apart from some scattered notes in other volumes of *Notes Ptéridologiques*); POSTHUMUS's records of ferns from that region, which are included in his work on the ferns of Surinam (1928), were partly based on Bonaparte's work, but comparatively little new material had been added to the existing collections. Consequently, when Mme M. Tardieu-Blot informed me that the Paris herbarium contained some unidentified collections of Pteridophytes from that region, I accepted willingly her offer to study them.

In this paper are enumerated new or critical or in some other respects interesting records of ferns from the material concerned. It is regrettable that most collections do not possess indications of precise localities, or even lack collectors names, numbers, or both; it is supposed that most of these specimens have been collected by Leprieur.

Hymenophyllaceae

Trichomanes diversifrons (Bory) Mett.

Leprieur (?) 37. In my opinion this species, as commonly understood, is still an aggregate, even if the next species is segregated.

Trichomanes trollii Bergdolt

Without collector's name or number. Previously known from British Guiana, Surinam, and Bolivia.

Polypodiaceae

Adiantum cristatum L.

Three fronds, without data, only labelled Guyane française or Cayenne. Not previously known from the Guianas.

Adiantum killipii Maxon & Weatherby

The material at my disposal contained a duplicate of one of the collections cited as cotypes: Leprieur s.n. ("In declivis montium guyanae centralis Oyapok superius, juin 1823"), bearing the name *Adiantum intermedium* Swartz. It is somewhat aberrant by the smaller size of the fronds and segments and by the sori occupying only the upper and outer margin of the roughly parallelogrammic pinnules; also the stiff, curved or flexuose fibril-hairs of the lower surface are absent on several (esp. sterile) segments, being evidently rather caducous. The same is true for the hairs on the reflexed parts of the lobes.

Additional specimens: Leprieur 145, without loc.; Surinam: Hostmann 710 a [L], without exact locality (first record from that country).

Adiantum latifolium Lam.

Certain specimens, as, for instance, Mélinon 540, and Tulleken 423 from Surinam [U], are in general habit intermediate between *A. latifolium* and *A. killipii*. The internodes of the rhizome are shorter than in the first and longer than in the second-named species; in texture they are thinner than *latifolium* usually is and resemble in that respect the specimens of *A. killipii* I examined; but they differ from that species by the absence of the characteristic fibrils of the lower side. In aspect they agree with pl. 23 fig. 14 of *Flora Brasiliensis* Vol. I part II. I include them tentatively in *A. latifolium*, but they may represent a separate species or variety. They have often been called *Adiantum intermedium* Sw., e.g. in *Flora Brasiliensis*, l.c., but the true identity of this often misunderstood species was pointed out by CHRISTENSEN (1910), p. 9.

Adiantum melanoleucum Willd.

A very large, at the base fully tripinnate, frond, without collector's name, number, or locality. It appears to be very much like *A. urbanianum* Brause, Fedde Rep. 15, p. 93, 1918/19, which I have not seen, referred to *A. melanoleucum* in Christensen's Index (suppl. I); perhaps it constitutes a distinct variety of this, although very variable, species. It has up to now not been recorded from the Guianas; as there is no label with indication of origin, one might doubt whether it really came from French Guiana. The matter is discussed at the end of this paper.

Adiantum serrato-dentatum Willd.

Some of the specimens of this species, which seems to be quite common in Guiana, e.g. Leblond 9, Leprieur 153 and 332, have partly larger pinnules (up to 11 mm long and 6 mm broad) with margins meeting under nearly right angles. They match exactly *A. rectangulare* Lindman (1903, p. 204; t. 9, fig. 3) from Matto Grosso. LINDMAN himself observed that "... Es ist möglich, dass diese Pflanze

mit dem *Ad. obtusum* var. *kunzei* Miquel identisch ist". If this is true, Miquel's name has priority, as it has been published originally as a species (MIQUEL 1843, p. 189), and was only later reduced by Baker to a variety of *A. obtusum* (HOOKER-BAKER 1874, p. 119: *A. obtusum*, Desv.; . . . β , *A. Kunzei*, Miquel . . .). Lindman's name would therefore be superfluous. After having compared Miquel's type (Focke 86, Para, Surinam [U]) with Lindman's figure and description, I am certain that they are identical. The question then arises as to the status of this taxon. It is very near *A. serrato-dentatum* Willd. (*A. obtusum* Desv.); although the type frond looks somewhat different by the crenato-lobate margins and the small number of segments per pinna (about 10), these are characters to be met with in otherwise typical *A. serrato-dentatum*, especially in sterile or incompletely fertile fronds. Moreover, Leblond 9 and Leprieur 153 contain other fronds which are indistinguishable from that species. Although these fronds are not actually attached to the same rhizome as those matching Miquel's *A. Kunzei*, it may be assumed that they belonged to one plant, since it would be quite fortuitous if they both consisted of a mixture of these two forms growing together. On the other hand, Lindman's (1903, p. 204) statement on *A. obtusum* Desv.: "Die Art scheint mir sehr konstant und monotypisch zu sein, weshalb ich keinen Anstand nehme, die folgende [*A. rectangulare* spec. nov.] als selbständige Art zu betrachten . . ." is not to be neglected, as Lindman reported to have seen a large number of specimens of the first species. I prefer therefore to follow BAKER in treating *A. kunzei* as a variety of *A. serrato-dentatum*, which must then be called *A. serrato-dentatum* Willd. var. *kunzei* (Miq.) Kramer comb. nov. — *A. kunzei* Miq. (1843), p. 189; *A. rectangulare* Lindman (1903), p. 204; *A. obtusum* Desv. var. *kunzei* (Miq.) Baker (1874), p. 119.

Another variety of this species, described as *A. obtusum* Desv. var. *revolutum* (errore: var. *revoluta*) by MIQUEL (l.c.), p. 188 (Focke 611, Surinami ad Osembo in Para. Apr. 1842, type [U]) seems not to be separable from *A. serrato-dentatum*. It consists of a sterile and an incompletely fertile frond, where the few sori seem to be continuous by the revolute margin, which Miquel apparently mistook for "singulo dente soro semilunari". POSTHUMUS cited the specimen under *A. serrato-dentatum*, in my opinion correctly.

Adiantum tetraphyllum H. B. Willd.

As usually understood and construed here, this species is probably an aggregate. Moreover, I am not sure that even part of the specimens belong to the true *A. tetraphyllum*, as this species was described (WILLDENOW 1810, p. 441): "... pinnulis oblongis, dimidiatis basi truncatis, margine superiore et apice oblique truncato . . .", and in all the Guiana material referred to this species that I examined the apices of the pinnules are rounded or somewhat narrowed, but never obliquely-truncate. Compare also MAXON's description (1926, p. 423): "... the fertile ones . . . the acutish or acuminate tip; . . . sterile pinnules . . . long acuminate, and falcate . . ."

A form which is probably separable differs by its very dark colour of the leaf-tissue and in the pinnules being very close, contiguous or even overlapping, and evenly parallelogram-shaped; this is *A. macrocladum* Klotzsch sensu Jenman (1898, p. 89). It is remarkable that neither Jenman's nor Hooker's (1858, p. 49) descriptions agree entirely with the original one, nor with one another. Without knowing the variability of the species, nor having seen the type, I prefer to leave the solution of the matter to a monographer; all these forms are included here in *A. tetraphyllum* in its broadest and collective sense. Specimens seen: Leprieur 76, id. 264: "in sylvis montosis ad flumen Oyapok", and Sandwith 1567 from British Guiana, Mazaruni Station, called *A. cayennense* Willd., but with hardly incised margins; also no. 1088 from Surinam, without collector or locality (both U).

Key to the species of **Adiantum** of French and Dutch Guiana:

1. Veins free or occasionally anastomosing 2
Veins copiously anastomosing; fronds herbaceous, with oblong pinnae **A. adiantoides** (J. Sm.) C. Chr. (FG)
2. Rachis almost dichotomously branched (i.e. flexuose, with alternate pinnae, their rachises being nearly as strong as the main one) **A. leprieurii** Hook. (FG, DG)
Fronds simply pinnate 3
Fronds bi- or tripinnate 9
3. Midribs black at the base of the pinnae for 1 cm or more **A. phyllitidis** J. Sm. (DG)
Midribs with a very short black portion at the base or pale throughout or indistinct 4
4. Lowermost pinnae with a very broad truncate-subcordate base, subsessile, 2-4 cm broad, subacute to shortly acuminate, mostly with a short black portion at the base of the midrib **A. macrophyllum** Sw. (DG)
Lowermost pinnae narrower, or, if up to 2 cm broad, with a much narrower base and long-acuminate. Midribs everywhere pale or indistinct 5
5. Petiole slender (0.3-0.5 mm at base of lamina); lamina ca. 6-12 cm long 6
Petiole strong (1 mm or more at base of lamina); lamina mostly longer 7
6. Lamina with a large terminal pinna; lower surface glaucous **A. petiolatum** Desv. (FG, DG)
Lamina without terminal pinna; rachis elongate, often rooting; Lamina without terminal pinna; rachis elongate, often rooting; lower surface not glaucous **A. deflectens** Mart. (FG)
7. Sori continuous 8
Sori distinct **A. obliquum** Willd. (FG, DG)
8. Midrib median. Pinnae 2-3 cm broad, up to 5(-6) at a side. Veins mostly somewhat anastomosing. Lower base of pinnae often narrowed-rounded (fronds always simply pinnate) **A. dolosum** Kze. (FG?, DG)

- Midrib mostly diagonal. Pinnae (or, in bipinnate fronds, pinnules) 1–2 cm broad, 6–18 at a side. Lower base of pinnae mostly narrowed-cuneate. Veins often completely free **A. lucidum** (Cav.) Sw. (FG, DG)
9. Sori continuous (or sometimes slightly interrupted by crinks of the margin) 10
Sori distinct 13
10. Pinnules dimidiate 12
Pinnules not dimidiate 11
11. Pinnules elliptic to roundish, blunt; blades freely bipinnate; petiole up to 60 cm long. **A. oyapokense** Jenm. (FG)
Pinnules lanceolate, sometimes acuminate; only lowermost pinnae with a few free segments; petiole shorter **A. lucidum** (Cav.) Sw. (FG, DG)
12. Sori on the basal half to two-thirds of the upper margin of the pinnules; these about 1 cm long **A. pulverulentum** L. (FG, DG)
Sori on the upper and outer margin of the pinnules; these about 2 cm long **A. villosum** L. (FG, DG)
13. Rachises glabrous; pinnules glaucous below 14
Rachises more or less fibrillose-tomentose 15
14. Sori deeply concavely curved; lamina often tripinnate at base **A. melanoleucum** Willd. (FG)
Sori not or hardly concave; lamina bipinnate throughout **A. glaucescens** Kl. (FG, DG)
15. Pinnules with a distinct diagonal midrib 16
Pinnules dimidiate, i.e. with the midrib running along the lower margin, or midrib very short or indistinct 17
16. Rhizome with long internodes; blades freely bipinnate **A. latifolium** Lam. (FG, DG) ¹⁾
Rhizome with short internodes; stipes clustered; blades with a few pinnate pinnae at the base. **A. obliquum** Willd. (FG, DG)
17. Apex of pinnae acuminate or acute; pinnae $1\frac{1}{2}$ –3 cm long **A. hirtum** Splitg. (FG, DG) ²⁾
Apex of pinnae blunt or rounded, or pinnae shorter 18
18. Rachises (at least the secondary ones) on both sides fibrillose-tomentose. 19
Rachises glabrous below, densely brownish-tomentose above; sori small; segments close **A. tomentosum** Kl. (FG, DG)
19. Leaf-tissue of lower side bearing permanent hair-like, narrow fibrils 20
Leaf tissue of lower side glabrous or glabrescent 21

¹ I cannot separate *A. argutum* Splitg., sometimes considered as a variety of *A. latifolium*; the type is in the Leiden herbarium.

² JENMAN (1898, p. 89) described a variety of *A. macrocladum* Kl. "with the margins deeply cut and the sori on the tips of the lobes, apart, like projecting beads along the margins, the pinnules being not rounded but much longer and acuminate, the colour equally dark.—Brasil and Peru." This I strongly suspect to be *A. hirtum* Splitg.

20. Fibrils of lower surface numerous, yellowish; pinnules small, mostly about 1 cm long, often folded when dried; texture thin **A. terminatum** Kze. (FG, DG)
Fibrils of lower surface fewer, dark brown; pinnules larger; texture firmer . . . **A. killipii** Maxon & Weatherby (FG, DG)
21. Pinnules small, rhombic, parallelogrammic, or roundish, rigid to coriaceous 22
Pinnules oblong-lanceolate; texture soft, $\pm$ herbaceous . . 23
22. Petiole rough; apex of fertile pinnules acutish or blunt; lamina often tripinnate at base **A. cristatum** L. (FG)
Petiole smooth; apex of fertile pinnules rounded; lamina always bipinnate **A. serrato-dentatum** Willd. (FG, DG)
23. Barren margin grossly crenato-dentate; sori on top of the crenations, separated by incisions
. **A. cayennense** Willd. ex Kl. (FG, DG)
Barren margin serrate; sori on the margin, not separated by incisions **A. tetraphyllum** Willd. sens. lat. (FG, DG)

Asplenium haplophyllum Domin

Leprieur 109, without exact locality. This species was originally described (DOMIN 1929, p. 170) from Amazonian Brazil and British Guiana. Mr. C. V. Morton kindly informed me that, as far as he remembered, it has also been collected in Colombia. The specimen cited agrees very well with Domin's description and figure, the terminal segments being only somewhat more distinctly serrato-crenate.

Bolbitis guianensis (Aubl.) Kramer comb. nov. — *Polypodium guianense* Aubl., *Leptochilus guianensis* (Aubl.) C. Chr., *Lomagramma guianensis* (Aubl.) Ching

This species was transferred to *Lomagramma* by CHING (Am. Fern J. 22, 1932, p. 17, not seen); HOLTUM (1937, p. 196) showed that its proper place is in *Bolbitis*, not in *Lomagramma*. As he did not make the new combination, it will be necessary to do so here. Specimen examined: Leprieur s.n., 1836, "in sylvis paludosis truncis arborum scandens ad ripas Juipi et Inini".

Bolbitis semipinnatifida (Fée) Alston

Mélinon 364, 1842, without exact locality. Not previously known from French Guiana, but recently collected in Surinam, and, according to ALSTON (1932, p. 310), known from British Guiana, Trinidad, and Venezuela.

A specimen labelled only "367" belongs either to this species or to *B. aliena* (Sw.) Alston.

Dennstaedtia ordinata (Klf.) Moore

Leprieur 133, without further data. The rather fragmentary material, consisting of pinnae or parts of them, and pieces of rachises, was identified with MAXON's key (1926, p. 491), and agreed well

with material from Venezuela in the Leiden herbarium. It is therefore fairly certain that it belongs to this species, characterized by sessile pinnae and pinnules, more or less truncate segments, and the pubescence of rachises and costae. It is noteworthy that no other species of the genus has ever been collected in the Guianas, except *D. adiantoides* (H. B. Willd.) Moore cited by POSTHUMUS (p. 68) also from French Guiana, which I did not see. Posthumus might have mistaken *D. ordinata* for *D. adiantoides*.

Diplazium arboreum (Willd.) Pr.

MAXON is followed here in uniting this species with *D. shepherdii* (Spr.) Link. Leprieur 45, without locality.

Diplazium expansum Willd.

Leprieur 117 (the herbarium label of Paris bears the number 353) and 43, both without further data. Greater Antilles, Northern South America to Brazil, but not previously known from Guiana. Possibly an aggregate, as was pointed out by MAXON (1926, p. 443).

Diplazium celtidifolium Kze.

The specimens which are placed here in this species show a very wide range of variation. Several of them: Leprieur 62, 116, 343, and an unnumbered collection "les bords de l'Oyapok vers ses sources" have relatively short fronds with short (up to 9 cm), coriaceous pinnae, the lower ones abbreviated and deflexed, some of the fertile ones being only $1\frac{1}{2}$ cm broad, with nerves once or twice forked, and predominantly or even entirely diplazioid sori. The sterile fronds of Leprieur 116 are peculiar in having pinnae with a truncate base (in a right to acute angle) above, this being more oblique in the lower ones, but also hardly rounded; but other fronds on the same rhizome show the shape which is characteristic in other Guiana specimens, with a rounded, often slightly auricled upper base with the margins meeting under an angle larger than 90° . If it were not for these fronds being attached to the same rhizome, one would be inclined to take them for two or even three different species. They are more coriaceous than usual, but represent possibly only a form of a dry and/or sunny habitat of *D. celtidifolium* in a broad sense. Another frond labelled only "Cayenne" approaches in texture and shape of pinnae *D. grandifolium* (Sw.) Sw., but is also referred here to *D. celtidifolium*, until a monographic treatment of this very complex group elucidates the status of all the forms.

Dryopteris (*Glaphyopteris*) *decussata* (L.) Urb.

CHRISTENSEN (1913), p. 160, and POSTHUMUS (l.c.) p. 43 cite a collection of Leprieur without further data. An additional specimen is Leprieur, 1847, s.n. "ad torrentes montis Tibourou". These are apparently the only collections of this species from the Guianas.

Dryopteris (*Ctenitis*) *protensa* (Afz.) C. Chr. var. *dicksonioides* (Fée) C. Chr.

A second specimen besides that cited by CHRISTENSEN (1920), p. 93, and POSTHUMUS, is Leprieur (?) 108. The var. *funesta* (Kze.) C. Chr. is much more common in Guiana.

Dryopteris (Stigmatopteris) sancti-gabrielii (Hook.) O.Ktze.

Leprieur 41, "in sylvaticis confertis montium guyanae centralis summo". Not previously known from French Guiana, but collected in British and Dutch Guiana, and besides in Amazonas, Trinidad, and Venezuela.

Dryopteris (Goniopteris) subtetragona (Link) Maxon

Leprieur without data, 1838, id. 183, without loc., p.p. Not previously reported from French Guiana.

Elaphoglossum glabellum J. Sm.

Leprieur 50. POSTHUMUS cited this species without having seen a specimen, only quoting Fée.

Elaphoglossum longifolium (Jacq.) J. Sm.

Leprieur (?) 122, without data. Not previously known from French Guiana. For the name of this species, that has often been called *E. rigidum* (Aubl.) Urb., see ALSTON (1932), p. 316.

Elaphoglossum schomburgkii (Fée) Moore

Leprieur 241, 242, 243, "ad truncos vivos arborum riparum summo fluminis Oyapok". This species is construed here in its broadest sense, as I am not able to draw good border lines between the closely allied forms; it includes the specimens from Surinam named *E. pteropus* C. Chr. by POSTHUMUS, p. 151, which they are certainly not. The cited collections belong to the form called *E. luridum* (Fée) Christ by CHRIST (1899). The true

Elaphoglossum pteropus C. Chr.

has also been collected in French Guiana: Leprieur s.n. "in truncis arborum, in sylvis aeternis ad amnem Gabaret"; it is as yet not known from Surinam.

Eschatogramme desvauxii (Kl.) C. Chr.

All the Surinam specimens, which were included by POSTHUMUS in *E. furcata* (L.) C. Chr., belong to this species. The same is true for the French Guiana specimens seen: Mélinon 383, Leprieur 103.

Nephrolepis exaltata (L.) Schott

POSTHUMUS apparently has not seen any material from French Guiana, as he cites (l.c., p. 66) only a reference to Aublet. Specimen examined: Leprieur 34, "stipitibua palmarum ad tipas Oyapok".

Polypodium (P.) dissimile L.

Leprieur 92. Not previously known from French Guiana, but

collected in Surinam, and, according to ALSTON (in litt.), in British Guiana.

Polypodium (*Microgramma*) *fuscopunctatum* Hook.

Leprieur 77, without loc. The first record of this species from French Guiana; but, as one of the specimens from Surinam cited by POSTHUMUS under *P. thurnii* Bak. really belongs to *P. fuscopunctatum*, it might have been overlooked. Apart from several other collections from Surinam, it is known from British Guiana and Ecuador (the type locality).

Polypodium (*Campyloneuron*) *lapathifolium* Poir. (*laevigatum* auct. non Cav.)

Leprieur 81, without exact locality. Not previously recorded from the Guianas.

Polypodium (*Phlebodium*) *leucatomos* Poir. (*decumanum* Willd.)

Leprieur (?) 93, without loc. The specimen (part of a lamina in a rather poor state) has distinctly laxly undulate-crenate margins and may belong to a distinct variety.

Polypodium ("Ctenopteris") *mollissimum* Fée

Leprieur s.n., "corticibus arborum ad ripas amnis Inipi"; not previously known from French Guiana, but, as MAXON & MORTON (1948), p. 77, pointed out, probably confused by POSTHUMUS with *P. cultratum* Willd.

Polypodium (*P.*) *plumula* H. & B. ap. Willd.

Leprieur (?) 95. Not yet recorded from French Guiana; included by POSTHUMUS in *P. hygrometricum* Splitg.

Polypodium (*Marginaria*) *polypodioides* (L.) Watt

All the Guiana material seems to belong to the var. *burchellii* (Bak.) Weatherby from tropical South America. Leprieur 535, id. (?) 101, Rech 12.

Polypodium (*Xiphopteris*) *trichomanoides* Sw.

POSTHUMUS united three species under this name: *P. taenifolium* Jenman, *P. nanum* Fée and *P. trichomanoides* Sw. s. str. The latter is in South America only known from French Guiana (collections examined: Rech 38, Leprieur s.n. "summo arborum muscis intermixtum in sylvis humidis Oyapok superius", a mixture with *P. taenifolium* and *nanum*), its area being predominantly West-Indian. The material from Surinam belongs to the two other species respectively. Another collection of

Polypodium nanum Fée

is Leprieur (?) 140. For the differences between the three species see MAXON (1913) and COPELAND (1952).

Polytaenium brasilianum (Desv.) Bened.

POSTHUMUS included this species in his key of "*Anthrophyum*", but did not give any further data. Leprieur 110, "ad truncos arborum, in sylvis ad ripas amnis Yavé adnatum".

Polytaenium jenmani (Bened.) Bened.

This species, which was described by BENEDICT (1907) as *Antrorhyum* and later transferred by him to *Polytaenium*, has been neglected by POSTHUMUS. BENEDICT cited as a cotype the specimen I saw: Leprieur 109, "rupibus humidis, in sylvis umbrosis circa Tacourou Epirok sitis in guyanae centrali".

Pteridium aquilinum (L.) Kuhn ssp. *caudatum* (L.) Bonap.

When identifying the material with TRYON's key (1941), I became convinced that two specimens (Leprieur 56, and one without data) belong to the var. *caudatum* rather than to var. *arachnoideum* (Klf.) Herter, to which up to now all Guiana material was referred, whereas one specimen (Leprieur 121) is intermediate between the two varieties. The first has, according to TRYON, a circum-caraibic area, its easternmost station on the continent of South-America lying in Western Venezuela, whereas the second is very widespread in tropical America; to this belongs Leprieur 55.

Pteris altissima Poir. (*kunzeana* Ag.)

POSTHUMUS did not see material from French Guiana. Leprieur (?) 57, without further data, and 101, "in paludosis ad basin montium guyanae centralis".

Pteris biaurita L.

Martin s.n., Cayenne, p.p.; Leprieur 99, "in sylvis humidis herbosis ad ripas rivuli Cacao, amnis la Comté"; Leprieur 119 and 120, without localities.

Pteris quadriaurita Retz.

Martin s.n., Cayenne, p.p.

This species, construed here in a rather broad sense, is a new record from French Guiana.

Vittaria gardneriana Fée

Leprieur 125, without exact locality; 145, id., and 103, "ad truncos arborum in sylvis guyanae centralis ad amnem Erepousinghe". Identified with Benedict's key (though the fronds are very short; up to 9 cm in one, up to 15 cm in another specimen) I am convinced it belongs here. Mr. C. V. Morton confirmed my opinion. BENEDICT cited it from Mt. Roraima in British Guiana; it is not known from Surinam.

Parkeriaceae*Ceratopteris*.

POSTHUMUS, who overlooked Benedict's preliminary revision of the genus (1911), called all specimens *C. thalictroides* (L.) Brongn. I saw the following species from French Guiana:

C. deltoidea Bened. Leprieur 39.

C. pteridoides (Hook.) Hieron. Leprieur (?) 380; id. 19.

An unnumbered specimen belongs perhaps to *C. thalictroides* (L.) Brongn. s. str., but this is not certain, as it is sterile. The last-named species, mentioned by BENEDICT in the New World from Jamaica only, seems to be more widespread; it was collected several times in Surinam.

The fourth species recognized by BENEDICT, *C. lockharti* (Hook. & Grev.) Ktze., and mentioned by him from French Guiana, was not present in the material at my disposition.

Cyatheaceae

Cyathea circumdentata Kramer nom. nov. — *Hemitelia leprieurii* Jenman (1898), p. 47, non Kunze, which is *Cyathea leprieurii* (Kze.) Domin, Pteridophyta (1929), p. 264 (not seen), considered a synonym of *Cyathea* (*Hemitelia*) *spectabilis* (Kze.) Domin by Posthumus.

This species is, in my opinion, not closely allied to *C. macrocarpa* (Pr.) Domin, as was stated by POSTHUMUS, but nearer to *C. multiflora* J. Sm., near which it was placed by JENMAN, although he states: "this is a very distinct species from multiflora . . .", the most conspicuous difference being the very distinctly crenate-serrate margins of the segments.

An additional specimen, besides the type, which I have not seen, is Poiteau, without number or locality, 1824; it differs from Jenman's description only by the presence of very short and small strigose hairs on veins and leaf tissue of the lower side. In this respect it agrees with a specimen from British Guiana (A. C. Smith 2873), identified by Mr. C. A. Weatherby. As these hairs give the impression of being somewhat caducous, JENMAN may not have found them.

Cyathea escuquensis (Karsten) Domin

Leprieur, without number or locality. The material differs from Karsten's description by the presence of (more) numerous bullate scales on the costules below; from MAXON's (1926, p. 388) by the absence of glandular pubescence below, which is, however, not mentioned by KARSTEN. Size, cutting, indusia, spores, and paraphyses agree well with KARSTEN's description and figures. The species was not yet known from Guiana; it was described from Western Venezuela, and later reported from Puerto Rico.

Cyathea multiflora J. Sm.

According to ALSTON (1932, p. 308), POSTHUMUS misinterpreted this species; he segregated *Hemitelia guianensis* Hook. I am not able to give a judgment on the problem, so I keep the material concerned provisorily under this name. Anyhow, the combination *Cyathea guianensis* is already preoccupied by *C. (Alsophila) guianensis* (Regel) Domin, (1930), p. 120.

Cyathea parkeri (Hook.) Kramer, comb. nov.

In accordance with ALSTON (1932, p. 308), this is regarded as a separate species. When *Alsophila* and *Hemitelia* are sunk in *Cyathea*, a new specific epithet becomes necessary, as Alston's name *Hemitelia strigosa* (J. Sm.) Alston is not available because of *C. strigosa* Christ 1897. The oldest name is *Alsophila weigeltii* Roemer ex Presl, 1836, but this was published as a nomen nudum. Therefore the epithet *parkeri* Hooker 1844 should be used.

Hemitelia hirsuta (Desv.) Weatherby

Leprieur s.n., "in sylvis umbrosis, ad amnem Conana secus rivulos, 1847". Identified by comparison with excellent material from British Guiana, (leg. A. C. Smith), named by Weatherby. Probably not previously known from French Guiana. When transferred to *Cyathea*, the species needs a new specific epithet, as its name is preoccupied in that genus by *C. hirsuta* Pr. (= *armata* (Sw.) Domin = *Alsophila swartziana* Mart.) and *C. hirsuta* Bak. (= *hirsutifrons* C. Chr.). Without having seen the type, nor Weatherby's paper containing the new combination in *Hemitelia*, I abstain from renaming the species.

Dubious specimens:

Several specimens could not be named satisfactorily. Leprieur 205 belongs to what is called the group of *Alsophila armata* Pr.; Sampaio's key (1925) leads to that species. That is, however, restricted to Jamaica and Cuba; the name *armata*, too, is incorrect, as was shown by MAXON (1922). There are several closely allied species of the group in South America, but I could not identify the material with one of them. The same holds for Mélinon s.n., Cayenne; this is probably the species of Sampaio's pl. 18. Both species are evidently new for the flora of French Guiana.

Marattiaceae*Danaea nodosa* (L.) J. E. Sm.

Mr. C. V. Morton drew my attention to the fact that the material from Surinam called *D. elliptica* Sw. belongs really to *D. nodosa*, characterized by its nodose rachis and nodeless petiole. Specimens from French Guiana are Leprieur s.n., 1838; Leprieur 275, cited by POSTHUMUS under *D. elliptica*, presumably belongs also to *D. nodosa*.

General remarks.

Among the species not previously known from French Guiana, the most outstanding are *Adiantum cristatum* L. and *A. melanoleucum* Willd., as these species are otherwise entirely West-Indian in their distribution. One might doubt the correctness of the locality; as the labelling in those days often was, and in the specimens concerned actually is, very incomplete, one might assume that the locality French Guiana is incorrect; they could for instance have been collected on French West-Indian islands, which doubtless have often been visited by the same ships that sailed to or from Guiana. This is probably true for two sheets belonging to *Dryopteris* (*Ctenitis*) *subincisa* (Willd.) Urb., bearing neither number nor locality; this species has never been collected in the Guianas, but it is known from Trinidad, whose flora is more akin to that of the Guianas than that of the other Lesser Antilles; but at present it seems better not to include it in the fern flora of French Guiana. I do not think, however, that the same should be assumed in the case of the two *Adiantum*'s; there are, in fact, not a few fern species in the Guianas whose distribution is predominantly or entirely West-Indian, but whose indigeneousness in the Guianas is not to be doubted, e.g. *Polypodium trichomanoides* Sw. and *P. taenifolium*, *Cyathea pungens* (Willd.) Domin, *Paltonium lanceolatum* (L.) Pr., etc. As far as I could ascertain, these species are, at least in Guiana, entirely montane.

I wish to express my sincerest thanks to Mme M. Tardieu-Blot and Prof. H. Humbert for sending me the collection (almost 500 specimens) on loan, to Mr. C. V. Morton for his helpful and critical remarks on some dubious specimens, and to the Director of the Leiden Herbarium for lending me valuable material for comparison.

REFERENCES

- ALSTON, A. H. G. 1932. Contributions to the flora of tropical America XIII. Pteridophyta collected by the Oxford Expedition to British Guiana, 1929. Kew Bull.
- BAKER, J. G. 1870. Cyatheaceae and Polypodiaceae, in *Flora Brasiliensis* I², Monachii.
- BENEDICT, R. C. 1907. The genus *Antrophyum*. I. Synopsis of subgenera, and the American species. Bull. Torr. Bot. Cl. 34.
- BENEDICT, R. C. 1909. The genus *Ceratopteris*: a preliminary revision. Contr. N.Y. Bot. G. 126; (Bull. Torr. Bot. Cl. 36).
- BENEDICT, R. C. 1914. A revision of the genus *Vittaria* J. E. Smith. I. The species of the subgenus *Radiovittaria*. Contr. N.Y. Bot. G. 171 (Bull. Torr. Bot. Cl. 41).
- CHRIST, H. 1899. Monographie des Genus *Elaphoglossum*. Zürich.
- CHRISTENSEN, C. 1909. Ueber einige Farne in O. Swartz' Herbarium. Ark. f. Bot. 9: 11.
- CHRISTENSEN, C. 1913, 1920. A monograph of the genus *Dryopteris*. Part I: The tropical American pinnatifid-bipinnatifid species. København. Part II: The tropical American bipinnate-decompound species. København.
- COPELAND, E. B. 1952. The American species of *Xiphopteris*. Am. Fern. J. 42: 2 and 3.
- DOMIN, K. 1929. The Pteridophyta of the island of Dominica, with notes on various ferns from tropical America. Praha.
- DOMIN, K. 1930. The species of the genus *Cyathea* J. E. Sm. A preliminary list. Acta Bot. Boh. 9.

- HOLTUM, R. E. 1937. The genus *Lomagramma*. *Gardens' Bull.* 9: 2.
- HOOKE, W. J. 1858. *Species Filicum*, vol. II.
- HOOKE, W. J. & J. G. BAKER. 1874. *Synopsis Filicum* (2nd ed.).
- JENMAN, G. S. 1898. The ferns and fern-allies of the British West Indies and Guiana, 1. Trinidad.
- KARSTEN, H. 1862-1869. *Florae Columbiae terrarumque adiacentium specimina selecta*. Vol. II.
- LINDMAN, C. A. M. 1903. Beiträge zur Kenntnis der tropisch-amerikanischen Farnflora. *Ark. f. Bot.* 1.
- MAXON, W. R. 1916. *Polypodium trichomanoides* and its allies. *Contr. U.S. Nat. Herb.* 87: 7.
- MAXON, W. R. 1922. The North American species of *Alsophila* grouped with *A. armata*. *Contr. U.S. Nat. Herb.* 24: 2.
- MAXON, W. R. 1926. Pteridophyta of Porto Rico and the Virgin Islands.
- MAXON, W. R. & C. V. MORTON. 1948. Pteridophyta, in: B. Maguire, *Plant explorations in Guiana 1944, etc.* *Bull. Torr. Bot. Cl.* 75.
- MIQUEL, F. A. G. 1843. *Observationes botanicae de quibusdam plantis, quas in Colonia Surinamensi legit vir graviss. H. C. Focke. Versl. en meded. uitgeg. d. h. Kon. Ned. Instit. v. Wetensch. ("Het Instituut")*.
- POSTHUMUS, O. 1928. The ferns of Surinam and of French and British Guiana. Malang.
- SAMPAIO, A. J. DE. 1925. Contribuições para a nova Flora Brasileira. O. Gen. *Alsophila* R. Br. (1810) na flora brasileira (Cyatheaceas). *Arch. Mus. Nac. Rio de Janeiro* 25.
- TRYON, R. M., Jr. 1941. Revision of the genus *Pteridium*. *Contr. Gray Herb.* 134.
- WILLDENOW, C. L. 1810. *Species Plantarum* V., Berolini.

SYMBIOTIC NITROGEN-FIXATION IN NON- LEGUMINOUS PLANTS

I. PRELIMINARY EXPERIMENTS ON THE ROOT-NODULE SYMBIOSIS OF ALNUS GLUTINOSA

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INTRODUCTION

Whereas symbiotic nitrogen-fixation in root-nodules of leguminous plants is the subject of a wide-spread research in the field of botany, microbiology, biochemistry and agronomy, little is known about the nitrogen-fixation of root-nodules which occur in non-leguminous plants. This is even more regrettable considering that not only are these symbiotic phenomena extremely interesting in themselves but also that it is not unlikely that a better knowledge concerning the nitrogen-fixation of root-nodules of certain non-leguminous plants might give a clearer insight into the process of nitrogen-fixation in leguminous plants. It would not be the first time in Biology that a change of subject matter with its accompanying possibilities for comparative research would open up new prospects which would never have been discovered within the limitation of the old, well-known subject matter. Up till now, however, research on the symbiotic nitrogen-fixation in non-leguminous plants has not succeeded in increasing the knowledge of symbiotic nitrogen-fixation in general. The fact that literature on the subject of nitrogen-fixation in root-nodules of non-leguminous plants is scarce is not only, and perhaps not even primarily, due to a lack of interest on the part of those working on problems concerning the nitrogen-fixation. The explanation may

be found in the difficulties which are experienced directly at the beginning of the experiments on nitrogen-fixation in non-leguminous plants.

It has proved impossible up till now to cultivate the organisms responsible for the formation of these root-nodules with certainty *in vitro*. Whereas a symbiosis cannot be studied profitably if one does not succeed in observing the properties of the component organisms separately, everything should be done to conquer the difficulties of isolation of these endophytes. Should this prove impossible, however, one should at least try to obtain some insight into the cause of these difficulties.

SURVEY OF LITERATURE

The initial research on symbiotic nitrogen-fixation of the root-nodules of the alder was carried out by HILTNER (1896, 1904) and HILTNER and NOBBE (1904).

Owing to the fact that the nodule-bearing alder and the seabuckthorn can be grown in N-free nutrient solutions they had already concluded that a fixation of elementary atmospheric nitrogen occurred in these nodules.

KREBBER (1932) and ROBERG (1934) repeated these experiments, thereby convincingly demonstrating the fact that the growth of alder plants with root-nodules was excellent on N-free nutrient solutions, whereas comparable plants without nodules perish on those solutions owing to lack of nitrogen. The fact that nitrogen was indeed fixed was abundantly proved by the Kjeldahl analyses of v. PLOTTO (1941), whereas VIRTANEN and SAASKAMOINEN (1936) even observed the secretion of nitrogenous compounds out of roots. More recently FERGUSON and BOND (1954) obtained convincing evidence that the nitrogen is fixed in the nodules of the nodulated roots.

The only objection one might raise with regard to all these experiments is that they could not be carried out in sterile conditions, so that there is a possibility, although an extremely improbable one, that the nitrogen fixation was caused by contaminating organisms e.g. *Azotobacters* or *Clostridiums* which had also been inoculated.

The microscopic-anatomical picture of the nodules is very well-known owing to the descriptions of SHIBATA (1902), SHIBATA and THAHARA (1917) and, especially, of SCHAEDE (1933). The infection itself, though experimentally established (the symbiosis is not of a cyclic kind) has never been observed microscopically. In the recently infected cells the endophyte forms clusters of thin actinomycetic hyphae which continually infect the younger cells very near the top. In the older cells vesicular swellings occur which should probably be considered to be the beginning of a degeneration under the influence of the counter-attack of the plant. In the still older cells this counter-attack has been accomplished and nothing is left of the endophyte but a small lump of cell-walls. In some instances a few cells of the endophyte would survive this counter-attack and sub-

sequently multiply in the intercellulars as "bacteroids". SCHAEDE suggests that these bacteroids can leave the root, multiply in the soil and from there again infect new alder plants. It is not altogether certain whether these bacteroids really belong to the endophyte. It is also premature to conclude from the microscopic picture that the endophyte belongs to the actinomycetes. One should realise that one observes the endophyte in the cells of the alder-root, influenced by the defense reactions of these cells. Only cultures on known nutrient media could increase our knowledge regarding the systematic position of the endophyte. For further physiological research such cultures are also indispensable. Attempts hereto have been made and published by several investigators e.g. by PEKLO (1910), BOTTOMLEY (1912), SPRATT (1912), ZIEGENSPECK (1929) and BORM (1931). These authors did indeed isolate organisms but they made no attempt at all to establish the identity of the isolated organisms by their nodulating capacity or they tried to do so by unreliable methods. The fact that notwithstanding this the contentions of these authors are still of force is proved by the fact that one of the leading new books on soil microbiology WAKSMAN (1952) mentions BOTTOMLEY's assertion that the endophyte of the alder belongs to the *Rhizobium* group. KREBBER (1932), a more critical observer, is forced to the conclusion that the endophyte cannot be isolated on the media used by him. In 1941 VON PLOTTHO remarks that an actinomycete can always be isolated on certain media which is even capable of forming nodules, though a long time might elapse after the inoculation. This pronouncement has been criticized by BOUWENS (1943). She pointed out that infections could occur spontaneously, given the cultural conditions used by VON PLOTTHO, which might be responsible for the appearance of the nodules. Fruitless efforts at isolation were made by Miss BOUWENS herself. Generally speaking we are forced to the conclusion that, although many organisms have been isolated from the nodules of the alder, none of these organisms can definitely be identified as the endophyte.

PERSONAL INVESTIGATIONS

From the above it will by now be clear that until now it has by no means proved to be easy to isolate the endophyte of the alder so as to allow us to conclude unconditionnally that the isolated organism is identical with the endophyte. In order to be certain of this identity it is necessary to prove in an effective and convincing way the ability to form nodules. This may be done in two different ways. One way is by using cultures of the alder in open jars as VON PLOTTHO did. In that case one has to ascertain statistically by comparing non-inoculated control plants and by using a great many cultures whether the amount of nodules occurring after inoculation is significantly more than that resulting from spontaneous infection. Alderplants may also be grown under conditions such as to preclude spontaneous infections, so that one can be certain that each nodule can be formed only in

the presence of the endophyte in the inoculation matter. In order to ensure a successful infection it is imperative to use healthy plants and therefore not advisable to use completely sterile plants under cotton wool. It is sufficient to use plants whose roots alone are sterile.

a. METHOD TO BE USED WHEN WORKING WITH NON-STERILE CULTURES

The method used was in principle derived from KREBBER (1932), and was the same as applied by VON PLOTHO (1941). Only the nutrient solution according to V. D. CRONE was replaced by a diluted solution according to HOAGLAND, as this provided a better supply of iron. Alder seed is collected yearly in the beginning of December shortly before the alder fruits drop off. In order to preclude the possibility of infection of the seed with endophyte it is cursorily disinfected by shaking for 5 minutes with a 0.1 % (v/v) solution of bromium. Subsequently it is placed in a dish with water and left to germinate. After germinating, which lasts approximately 5 days, the seedlings are removed to a small glass container, the bottom of which is covered up to a height of ± 4 cm with a good nutrient solution containing $\pm \frac{3}{4}$ % of agar. After about 3 weeks, when the plants have grown to ± 4 –5 cm they are carefully pulled out of the soft agar and fixed with a piece of cotton-wool in pierced corks on jars containing 300 ml. In each jar three plants are placed. Then the jars are filled with the following nutrient solution: 1000 ml of water (dest.), 0.126 grams of KNO_3 , 0.295 gr. $\text{Ca}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$, 0.012 gr. $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 0.034 gr. KH_2PO_4 to which 1 ml of a saturated solution of ferritartrate and 1 ml of a A-Z solution of the following composition is added; 1000 ml of water, 2.86 gr. H_3BO_3 , 1.81 gr. $\text{MnCl}_2 \cdot 4\text{H}_2\text{O}$, 0.22 gr. $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$, 0.07 gr. Na_2MoO_4 , 0.08 gr. $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$.

This solution is renewed at regular intervals until the plants have reached a height of about 8 cm in total. Then the nutrient-solution is replaced by a solution of the same composition except for the fact that the nitrates have been replaced by equivalent quantities of KCl and CaCl_2 . Simultaneously the inoculation-matter (for instance nodule suspensions or isolated organisms) is added to the solution. During a week the jars are carefully shaken once a day, in order to ensure a firm contact between the endophyte and the roots. At the end of the week the solution is renewed by a N-free solution of the same composition, this time without adding inoculation matter. This renewal is repeated once a week until the nodules have grown sufficiently to be counted. This is generally the case after 6 weeks. It did not appear to be necessary to aerate the cultures. As BOUWENS (1943) has shown, this method may induce spontaneous infections. I doubt whether these are aerial infections. They are more likely caused by polluted surroundings. When the jars are placed on clean wooden tables and not on tablets with soil amongst other plants, and one takes care not to touch the roots with one's fingers, the chances of spontaneous infections occurring during brief experiments are practically nil.

b. METHOD ADOPTED WHEN WORKING WITH STERILE CULTURES

The method used for working with sterile cultures is essentially the same as that used for working with non-sterile cultures, differing of course only in the measures taken to ensure adequate sterility. First the seed has to be disinfected. After prolonged experiments by various means of disinfection (e.g. sublimate and concentrated sulphuric acid) the following complicated method proved to be the most satisfactory one. The seed is first shaken with soapsuds, thoroughly rinsed in water, washed for 4 minutes in alcohol 96 %, again rinsed in water, and then disinfected during 15 minutes with a 0.1 % (v/v) solution of bromium. Afterwards the seed is quickly rinsed in sterile water and then placed in a solution of 0.5 % (w/v) of hydroxylamine hydrochloride in water (according to BjÄLVE), see KYLIN (1950). Here it remains overnight and is thoroughly rinsed the following morning with 4 portions of sterile water.¹ Hereafter it is placed in small testtubes filled with sterile water and left to germinate. It is not advisable to let the seeds germinate jointly e.g. in a petridish as, in that case, possibly still infected seeds may infect the others. On the 5th day the germinated seeds are transferred to wide culture tubes filled halfway with a sterile Hoagland solution containing $\frac{3}{4}$ % of agar and 0.1 % of glucose, in order to be able to recognize infected plants more readily. Three weeks later the plants which have remained sterile are taken out of the tubes in a room disinfected by UV light and wrapped in a strip of sterile cottonwool at the level of the hypocotyle. Thereafter they are placed in tubes of the type shown in fig. 1 with a content of 100 ml. These tubes are thereafter filled with a sterile Hoagland solution via the little tube at the side. Subsequent renewals of the nutrient solution and the inoculation can also take place via this little tube. In order to prevent the contents of the tube from being infected by the no longer sterile leaves during this process the nutrient-solutions are administered by way of siphon flasks whose siphon has a filling apparatus at its extremity, as is shown also in Fig. 1. For long-time experiments the small culture-vessels are no longer suitable. In such cases one should use the culture in suction-Erlenmeyers, as described by LAINE and VIRTANEN (1941). The same drawback as the one already mentioned by GERRITSEN (1935) applies to this method viz. that the fastening of larger plants in the plug of cottonwool facilitates the penetration of aerial infections. Much less trouble is experienced during short-time infection experiments with rather smaller plants. I have never observed spontaneous nodulation to take place during this culture method. As with the non-sterile method the nodules can be counted six weeks after the inoculation has taken place. The appearance of numerous nodules after inoculation with a suspension of root-nodules is certain proof of the usefulness of this method.

¹ The best way to execute all these processes in floating seeds of the alder is to use separation funnels whose tapopening is sufficiently small to prevent seeds from passing through whilst the glassstopper has been replaced by a plug of cottonwool.

All the cultures, whether sterile or non-sterile, were placed in a small greenhouse on the roof of the laboratory which was heated in winter and sufficiently ventilated and screened in summer to keep the temperature from rising too much. During the period of 15 September – 15 March 4 TL fluorescent lamps, each of 60 W, were used for extra lighting from seven in the morning to nine at

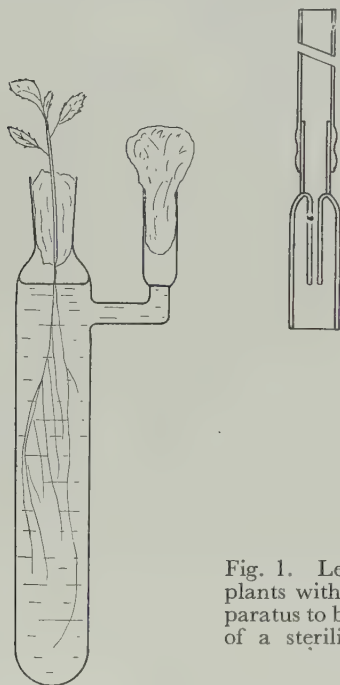


Fig. 1. Left: tube for cultivation of alder plants with sterile roots. Right: filling apparatus to be fixed at the end of the siphon of a sterilized siphon flask with culture solution

night. This enabled us to carry on with the culture of alder plants during the winter months also, although growth was retarded and the capacity to form nodules decreased. The factors which influence the capacity to form nodules will be more fully discussed in a later publication.

c. ENDEAVOURS TO ISOLATE THE ENDOPHYTE

In various ways we have tried to isolate the endophyte from the nodules of the alder:

1. freshly gathered young nodules were disinfected externally by shaking them for 4 minutes with a solution of 0.2 % sublimate in 0.5 % hydrochloric acid and subsequently 3 times rinsed in sterile water. In some instances the outer cell layers were removed at the same time with a sterile knife. Afterwards the nodules were pulverised in sterile water and then spread on nutrient media with 1.5 % agar. Incubation at 30° C.
2. After the same preliminary treatment as described under 1) the

nodules were also pulverised in sterile water. Then a series of dilutions was prepared out of this suspension and $\frac{1}{2}$ ml of each dilution was added to tubes containing 10 ml sterile nutrient media with 1.5 % of agar, which after melting were cooled to 40° C. After mixing thoroughly the tubes were emptied into sterile petri dishes and these incubated at 30° C.

3. After the same preliminary treatment as described under A (either only disinfection or peeling or a combination of both, whereby now and again a disinfection with a 0.1 % solution of bromium was used), the nodules as such or small fragments of these nodules were sown on nutrient media with 1.5 % of agar and incubated at 30° C.
4. Nodules which had had no preliminary treatment were pulverised in a drop of sterile water. Under the microscope the endophyte clusters were transferred with the aid of a micromanipulator to other drops of sterile water. This was repeated until the clusters were considered to be freed of attached impurities. Thereafter they were either incubated as hanging-drop cultures in liquid media or transferred on to media with 1.5 % of agar and incubated at 30° C.

With all methods a great deal of media were used. Besides the usual media as maltextract, yeastextract, broth or even simple tap-water, variations of the media, usual to the isolation of *Rhizobium* and also of the medium recommended by VON PLOTHO were mainly used. The composition of these media is as follows:

Rhizobium culture solution: 1000 ml of water, 0.5 gr. NH_4NO_3 , 0.5 gr. K_2HPO_4 , 0.2 gr. $\text{MgSO}_4 \cdot 7 \text{H}_2\text{O}$, 0.2 gr. NaCl, 2 gr. CaCO_3 .

Actinomycete culture solution according to VON PLOTTH: 1000 ml of water, 0.5 gr. NH_4NO_3 , 0.3 gr. KH_2PO_4 , 0.2 gr. K_2HPO_4 , 0.2 gr. $\text{MgSO}_4 \cdot 7 \text{H}_2\text{O}$, 0.1 gr. NaCl and 0.05 gr. CaCl_2 .

The variations in these nutrient solutions were provided by the choice of different sources of carbon (glucose 2 % or 0.1 %, mannitol 2 %, glycerol 2 %, starch soluble 2 % and 0.25 %, cellulose soluble 0.25 %, pectin 0.5 %), variations in the salt-composition (replacement of K_2HPO_4 by KH_2PO_4), omission of NH_4NO_3 , omission of CaCO_3 , dilution of the total salt concentration, addition of iron salts and A-Z solution, addition of organic nitrogenous substances (e.g. albumin 0.025 %, peptone 1 %, 0.5 % or 0.05 %, asparagine 0.1 %), additions of yeast autolysate, or extracts of yeast, broth, alder-roots, soil or peat.

The influence of the following substances was studied auxanographically during isolations according to the method 2 viz.: Ca-lactate, Na-acetate, Na-citrate, Na-succinate, K-tartrate, Ca-glycero-phosphate, hexosediphosphoric acid, casein and tannin.

The cultures were incubated under aerobic as well as under anaerobic conditions. Moreover we tried to obtain more subtle differences in redox-potential by auxanographic addition of agar-blocks with ascorbic acid, thiourea, Na-sulphite and K-permanganate to the agar media.

The results of all these experiments can be briefly summarized as follows:

Nowhere did an organism develop when method 4 was used. On applying the three other methods several organisms developed. Only the organisms which appeared with great regularity were kept and examined with regard to their nodulating capacity. During certain experiments when method 3 was used all organisms which developed out of a group of nodules were examined. None of these organisms proved capable of forming nodules whether the sterile or non-sterile method was employed. It should be noted that the organism isolated by VON PLOTTHO, which I received from the collection of the Central Bureau for Mould Cultures at Baarn, also proved incapable of forming nodules. It may be possible, however, that this organism has lost its virulence owing to the long culture *in vitro*. A more serious objection to the correctness of VON PLOTTHO's argument is the fact that I have never come across VON PLOTTHO's organism amongst the organisms isolated by me. On the strength of my own attempt at isolation I can only subscribe to BOUWENS' criticism and come to the conclusion that the endophyte cannot be isolated in this way. The assertions made that the endophyte is a *Rhizobium* or a certain actinomycete should therefore be considered of no value whatsoever.

It is well-known that several parasitical or symbiotically living organisms cannot be grown without their host. One has only to remind oneself of the obligate parasitically growing moulds or the endophyte of *Lolium temulentum*. These are, however, mostly organisms which are also never found in natural surroundings without their host-plant. It is difficult to accept the view that this is also the case with the endophyte of the alder. ROBERG (1934) observed on sowing alderseed on various soils which had never produced alders that nodules were formed all the same. The only way this can be explained is by accepting the view that the endophyte multiplies in these kinds of soil as a normal element of the microflora. We are justified by this in hoping that it will prove possible to find circumstances in which the endophyte will be able to multiply *in vitro*.

d. INVESTIGATION INTO THE CAUSE OF THE FAILURE OF THE ENDEAVOURS AT ISOLATION

It seemed rather pointless to proceed with our endeavours at isolation, as described in the previous chapter, without first having obtained some insight into the possible causes of these failures. These possible causes can be subdivided into 4 categories.

1. The endophyte contained in the nodules used for isolation is no longer vital after undergoing the necessary pre-treatment.
2. The endophyte can undoubtedly be grown on certain media, but during the isolation and the following growth *in vitro* it loses its virulence, so that its identity cannot be proved by the formation of root-nodules.
3. The type of growth of the endophyte is so slow, so diffuse, or so brief, that no visible colonies can be formed.

4. The endophyte requires special nutrition and/or environmental circumstances, which up till now have not been fully provided by the growth-circumstances and nutrient media used.

ad 1. The possibility that the endophyte contained in the nodules used for isolation purposes should no longer be vital can by no means be ignored. Examples of other symbioses are known. E.g. the bacterium causing the leaf-nodules of *Ardisia* cannot be isolated from these nodules, but this isolation is possible from the vegetation-top (e.g. see DE JONGH 1938). The so-called bacteroid stage of the *Rhizobium* in leguminous plants cannot develop after having been isolated with the micromanipulator on media where the *Rhizobium* is capable of showing marked development. (ALMON 1933). The nodules of the alder undoubtedly contain vital stages of the endophyte, otherwise these nodules could not be used for infection purposes. However, when the main body e.g. the vesicular stage would no longer prove vital and the vital cells would lie superficially close to the top, these vital stages would be quite likely to be either killed or removed by the pre-treatments (disinfection and peeling). Most authors, when using isolation methods took into consideration the possibility of the endophyte being killed by a too strong disinfection, but no one has proved experimentally whether this was indeed to be feared. This can be easily done by checking whether the nodules are still capable of forming nodules after undergoing pre-treatments.

Exp. 1. A number of freshly gathered nodules was divided in approximately equal parts. Each part was subjected to another pre-treatment. These pre-treatments consisted of disinfection with a solution of 0.2 % (w/v) HgCl_2 in 0.5 % HCl during 5 or 15 minutes, or with a solution of 0.1 % (v/v) bromium for 5 minutes, whether in combination with the removal of the outer cell-layers or not. Each group of nodules was then pulverised in sterile water and a quantity of these suspensions added to non-sterile alder cultures large enough to provide each jar with a quantity equivalent to 100 mg fresh weight of the original nodules. With each suspension 3 jars, each containing 3 plants, were inoculated. The result is shown in table 1.

TABLE 1 (Exp. 1)

The influence of the pretreatment of the nodules on the vitality of the endophyte

Means of disinfection	Length of disinfection	Peeling	Number of nodules produced per plant (average with stand. dev. of the average)
0.1 % HgCl_2	5 minutes	—	7.1 $\pm$ 1.3
0.1 % HgCl_2	5 minutes	after disinf.	14.6 $\pm$ 5.2
0.1 % HgCl_2	15 minutes	—	1.1 $\pm$ 0.4
0.1 % Bromium	5 minutes	—	18.4 $\pm$ 4.0
0.1 % Bromium	5 minutes	before disinf.	18.0 $\pm$ 1.0
Untreated	—	—	4.3 $\pm$ 1.3
Control (not inoculated) .			0

When considering the results of exp. 1 one must be sure to take into consideration the fact that the disparities in the observation series are not only the result of the disparities in pre-treatment, but also of those which originally existed between the groups of various nodules. At any rate these experiments prove that after all kinds of pre-treatments the nodulous matter was still clearly of an infectious kind. There is, therefore, no reason to suppose that failure of our endeavours at isolation was due to these pre-treatments.

On the strength of the microscopical research of the nodules it is reasonable to suppose that the vitality of the endophyte can be found only in certain parts of the nodule and that principally the vesicular cells, as also the bacteroids of the leguminosae, are not capable of further development. Whereas according to the description of the endeavours at isolation fragments of nodules were often used, the failures may perhaps partly be ascribed to the use of non-vital parts of nodules.

Exp. 2. A number of young root-nodules averaging a length of 2 mm and a total fresh weight of 1 gram was sliced at 1 mm distance from the top and thereby divided into a top- and basal fragment. Both series were pulverised in 10 ml of water each and a $\frac{1}{10}$ and a $\frac{1}{100}$ dilution was moreover made of these suspensions. Out of each dilution 4 jars containing 3 alders were inoculated with $\frac{1}{2}$ ml. The result may be seen in table 2.

TABLE 2 (Exp. 2)
Comparison of the vitality of the endophyte in various parts of the nodules

Fragment of nodule	Dilution of the suspension	Number of nodules produced per plant (average with stand. dev. of the average)
Topzone	undiluted	3.4 $\pm$ 1.4
	$\frac{1}{10}$ diluted	0.9 $\pm$ 0.4
	$\frac{1}{100}$ diluted	0.1 $\pm$ 0.1
Basal zone	undiluted	4.2 $\pm$ 1.5
	$\frac{1}{10}$ diluted	0.6 $\pm$ 0.2
	$\frac{1}{100}$ diluted	0

Contrary to all expectation there appears to be no difference either qualitatively or quantitatively in the capacity of both zones to induce infection. It would be premature to conclude from this that the vesicular stage is as vital as the "actinomycete" stage in the topzone, as hyphae may still be found between the vesicles also. We may however draw a practical conclusion from this experiment viz. that one need have no fear of using non-vital zones of the nodules during the efforts at isolation.

The experiments concerning the formation of nodules and the efforts at isolation are not entirely comparable inasmuch as regarding the first experiments the endophyte is able to contact the alderroots directly after suspension, whereas regarding the second experiments

this suspension is maintained during the incubation period. One may question whether the negative result of the efforts at isolation may not depend upon the dying off of the endophyte owing to the suspension in water or nutrient media.

Exp. 3. 2 grams of fresh nodulous matter was pulverised in 600 ml of water. This suspension was distributed over 6 Erlenmeyers of 100 ml, each of which therefore receiving 100 ml suspension. With HCl and NaOH the pH in 4 Erlenmeyers was regulated to resp. 3, 5, 8 and 10. The other two were set at pH 5 and conserved under anaerobic conditions over alcalic pyrogallol. One of these moreover was made extra-anaerobic by adding 0.1 % of cysteine. All these Erlenmeyers were thus kept at very different pH and Eh for 17 days at 27° C. At the end of this period of conservation 10 ml per jar of each Erlenmeyer were inoculated in 4 jars containing 3 alderplants. It is noteworthy to record that these concentrated nodulous suspensions were not spoiled by bacterium-or mould development during conservation (antibiotic influences e.g. on account of the high degree of tannin?). The result is shown in table 3.

TABLE 3 (Exp. 3)

Tenability of nodulous suspensions in water under various ways of conservation

Way of conservation		Number of nodules produced per plant (average with stand. dev. of the average)
pH 3	aerobic	73.6 ± 8.6
pH 5	aerobic	54.3 ± 9.8
pH 8	aerobic	58.2 ± 7.9
pH 10	aerobic	67.0 ± 8.5
pH 5	anaerobic	80.5 ± 9.5
pH 5	anaerobic + 0.1 % cystein	52.3 ± 10.1

A very large amount of nodules is formed in all cultures. We shall not discuss the cause of this high rate of nodules now, as we intend to discuss this in a later publication. At this stage it is only of importance to record that the endophyte loses none of its vitality owing to the conservation in suspension under any of the conditions, even those of rather extreme pH's and Eh's. There is no reason therefore to fear that, owing to suspension under conditions at variance with those in the nodules, the endophyte would be lost. The resistance to conservation even appears to be remarkably great.

One would almost suspect the endophyte to form resistant spores. There is no reason, however, to think this. This is namely proved by experiments during which I have subjected the nodulous suspensions to the influence of higher temperatures. The infectuous capacity of such a nodulous suspension was seen to have completely disappeared after heating the suspension for an hour at 60° C. This is indeed a very strong proof that no resistant spores are present. Furthermore,

it's logical conclusion in practise is, that one has to be careful during the pre-treatments in using high temperatures. It is better to avoid methods of disinfection during which the nodules are externally flamed e.g. with alcohol.

The above mentioned experiments might be criticized by pointing out that the nodules formed were not produced by the endophyte, but by a nodulating substance. On microscopical investigations, however, the nodules appeared to show the usual microscopical picture of cells filled with the endophyte. Moreover, after filtration through a Seitz bacterium filter the nodulous suspensions appeared to form no nodules whatsoever, whereas on the other hand the filter residue retained the same nodulating capacity as the non-filtrated suspension. This altogether rather improbable criticism seems to me to be adequately disproved hereby.

Summarising we may draw the conclusion that not a single reason exists to suppose that the inoculation matter used for isolation purposes would no longer contain the endophyte in a vital condition. The cause of the failing of the isolation endeavours mentioned sub. 1. cannot therefore be the real cause.

ad. 2. The infectuous capacity of several parasitically existing organisms is known to diminish after a certain period of culture in vitro. Cultures of *Rhizobium* also show similar symptoms, first in the form of a diminishing of the nitrogen-binding capacity of the formed nodules (efficiency), later as a diminishing of the nodule-forming capacity itself (virulence). It might be that the endophyte possesses this quality to such a marked degree, that the virulence disappears directly during the isolation. This would mean that one of the organisms, isolated up till now, is definitely identical with the endophyte but that one is unable to prove this identity as the isolated organism is not able to form nodules after the isolation. The following observation shows this to be untrue.

When trying to isolate the endophyte from a group of freshly gathered nodules according to method 3, after disinfecting and removing the outer cell-layers, the nodules are sown on a good nutrient agar (e.g. *Rhizobium*-salt solution with 2 % of glucose, 0.5 % of peptone and 1 % of yeast autolysate). Micro-organisms are seen to develop out of most nodules. None of these organisms, however, are able to form nodules after isolation. A small percentage of nodules (varying from 2–20 %), however, remains sterile. The question now arises whether these sterile nodules were indeed completely sterile at first or possessed the endophyte as did the other nodules. If this were the case one may expect on the strength of the experiments mentioned under *ad. 1.* that they will also still contain this endophyte in vital condition after the incubation period from 4–6 weeks. The last supposition proved to be true. When such "sterile" nodules are pulverised in sterile water and used for inoculation of sterile cultured alder plants several nodules may be seen to appear after a few weeks. By spreading the suspension simultaneously over various nutrient

media (broth agar, pepton-glucose agar, yeast agar etc.) this suspension may be observed to be truly sterile according to the usual microbiological criteria. Yet, notwithstanding this, the endophyte appears still always to be present in a vital condition.

When one of the organisms which had grown out of the other nodules would indeed be identical to the endophyte, one fails to understand why this endophyte should be produced by one nodule and not by the other, given the same medium and completely similar circumstances. Mechanical obstructions, such as impermeable cell layers, cannot affect this as no development is possible even after the "sterile" nodules have been pulverised and suspended in water. The most likely conclusion is that the organisms which were produced by the nodules had nothing in common with the endophyte. Failure up till now to carry out these isolation endeavours cannot be explained by, or imputed to, loss of virulence during the isolation.

ad. 3. During our endeavours at isolation as carried out by us up till now the appearance of a perceptible colony was taken as criterion for the occurrence of growth of the endophyte. In doing so heavy demands indeed are made upon the multiplication *in vitro* viz. that millions of cells of which a small colony already consists will develop out of the small quantity of cells present in the inoculation matter (and this specially applies to methods by which the inoculation matter is suspended in the medium or where micromanipulator-isolations are used). Possibly on certain media growth of the endophyte has really occurred, but has been too insignificant to lead to the formation of a visible colony. Certain microorganisms are also known to show a type of growth which does not at all produce visible colonies, and where the position of the colonies can only be recognised by the appearance of solution of suspended particles e.g. chalk, cellulose in the agar. The endophyte also might be able to show a similar type of growth on agar media. It is however unlikely that the failures are caused by this alone. E.g. if one examines the sterile nodules during the experiment mentioned under sub. 2 after 6 weeks incubation on agar media, one sees that although these nodules are markedly shrunken the microscopical picture of the endophyte remains the same as before incubation takes place. In the shrunken cells one can still observe the clusters of the endophyte without any indication of the endophyte extending its boundaries. This is not complete proof in itself as one cannot be certain even after the experiments discussed in chapter *d*, whether these clusters, and this especially applies to the vesicular stage, represent the most vital parts of the endophyte present in the nodules.

There are other reasons also why we should pay attention to this matter. However likely it may be that the failure of the isolation endeavours carried out up till now is mostly due to special food or environment requirements of the endophyte which the media used up till now have not supplied, the chance of success during the research will undoubtedly increase if one possessed a method to

determine the eventual growth of the endophyte in a more sensitive way than is possible on the strength of the formation of colonies on agar plates.

e. THE PREPARATION OF PURE INOCULATION MATTER

One of the greatest difficulties when working on the endophyte of the alder consists of the fact that not only does it prove impossible to let the endophyte grow *in vitro* but one even fails to liberate the endophyte from other micro-organisms. Also, after disinfection of the nodules combined with the removal under sterile conditions of the outer cell layers, several fungi, actinomycetes and bacteria appear to be present. None of these may be considered identical with the endophyte. This prevents working under sterile conditions. From the experiment described in chapter *d* under 2 we can now derive a method which enables us to acquire the endophyte in pure conditions. When one crushes in sterile water the nodules, which have remained visibly sterile after an incubation period of 6 weeks on a suitable nutrient agar, we acquire a suspension which is sterile according to the usual microbiological criteria, yet appears to possess the endophyte still in its vital, virulent form. Such matter, selected by incubation, is eminently suitable for use as inoculation matter in future growth experiments, and in general for work under sterile conditions. Although, to be sure, one has not got any pure cultures, such suspensions can be called pure suspensions.

f. DEMONSTRATION OF NITROGEN-FIXATION UNDER PURE CONDITIONS

Although the experiments by KREBBER and ROBERG show that very probably elementary nitrogen is fixed in the root-nodules of the alder, it seemed desirable in the first place to investigate this once again under pure conditions, now the possibility hereto existed. It was desirable moreover because it was important to know whether nodulous matter selected by incubation really contained the endophyte in a completely healthy condition, and whether especially during the incubation its efficiency was not impaired. To this purpose some sterile aldercultures were inoculated with pure nodulous suspensions, which had been prepared according to the method described in chapter *d*, ad. 2. As these experiments took rather a long time, the plants were conveyed a few weeks after the infection from the usual tubes to suction Erlenmeyers of 500 ml according to the method of LAINE and VIRTANEN (1941). Inoculation matter, as well as the alderroots, were examined with regard to the possibility of existing infections by spreading on a peptone-glucose agar and malt-agar. Only shortly before the end of the culture period some mould infections were seen which could, however, no longer influence the result. Directly at the first sign of these infections the experiment was terminated. The nitrogen analyses were carried out according to Kjeldahl by destruction with 12.5 ml of concentrated sulphuric acid, 750 mg Na_2SO_4 and 350 mg HgO , followed by distillation in a Parnas-Wagner apparatus in a 2% boric acid solution. The

nitrate-containing solutions were analysed by also heating them slightly in a Parnas-Wagner apparatus for $\frac{1}{2}$ an hour with Devarda metal (1 gr. per 50 ml solution), followed by distillation in a 2 % boric acid solution. The average nitrogen content per seed was analysed, the amount of nitrogen taken out of the nutrient solutions, (agar, nitrogen-containing solution and nitrogen-free solution) the amount of nitrogen in the inoculation matter and, at the termination of the experiment, the nitrogen content of the plants and the surrounding liquid. The result is given in table 4. 21 VI 51 was inoculated, 31 X 51 harvested.

TABLE 4

Nitrogen balance of two pure grown alderplants which were inoculated with pure nodule suspensions
(nitrogen expressed in m. aeq. N per plant)

	Plant 1	Plant 2		Plant 1	Plant 2
Present in seed	0.004	0.004	Found in plant	1.856	2.917
Taken from agar	0.010	0.015	Found in culture solution	0.024	0.005
Taken from nutrient solution	0.092	0.151			
Present in inoculation	0.001	0.001			
Present in N-free nutrient solution	0.002	0.002			
Nitrogen increase	1.771	2.749			
	1.880	2.922		1.880	2.922

The result is very clear. In both plants a convincing increase of nitrogen is observed. This proves that, elementary nitrogen can be fixed by the nodulated roots. Secondly, it proves that the nodules selected by incubation contain the endophyte in an efficient form.

DISCUSSION

Up till now it has not proved possible to cultivate the endophyte from the root-nodules of the alder apart from the nodules in vitro. Neither the attempts described in the literature, nor our own attempts which I made as an introduction to this investigation could produce any result at all. All pronouncements to be found in literature concerning successful attempts at isolation are unreliable, as the identity of the isolated organisms was either not established at all or was established with the aid of a method which could not be considered to be conclusive. Much attention was therefore paid by me to the developing of a method which would undeniably demonstrate the faculty of the isolated organisms of forming nodules. It proved impossible however to prove by this method the formation of nodules by one of the isolated organisms.

A detailed examination of the possible causes of the failure of these attempts at isolation was made. The inoculation matter used proved

to contain the endophyte still in a vital condition, so that the cause lay not herein. Both after the application of external measures of disinfection, such as disinfection with bromium or sublimate, as after removal of the outer cell layers the nodules proved still to be considerably infectious. Also after prolonged suspension in solutions of divergent pH and Eh the endophyte endured remarkably well. The endophyte only proved to be very sensitive to high temperatures. There is also no reason to suppose that one of the isolated organisms would be identical with the endophyte, but that it would have lost its virulence during the isolation and the following incubation, so that the identity of the isolated organism with the endophyte could no longer be proved with the aid of the capacity to form nodules. For nodules which stayed visibly sterile after remaining for 6 weeks on a good nutrient medium appeared to contain the endophyte at the end of this incubation period still in a vital form. The endophyte was therefore clearly not able to develop on this nutrient medium. For further development of the investigation there are two factors of considerable importance. By sowing nodules, after first having disinfected them and having removed their outer cell layers, on a good nutrient medium and, by selecting the nodules which have remained visibly sterile after an incubation period of 6 weeks, one can gather nodulous matter which is pure according to the usual microbiological criteria, but still contains the endophyte in a vital, virulent and efficient form. This inoculation matter, selected by incubation, enables one therefore to conduct experiments under sterile conditions. By means hereof the appearance of elementary nitrogen fixation under "sterile" conditions has been proved already. For further growth experiments of the endophyte similar pure inoculation matter may be likewise used.

Besides this it will be of great importance to elaborate a method which will enable us to demonstrate the growth of the endophyte in a more sensitive manner than was usual up till now. In the first place it is not certain that the endophyte will ever be able to grow so quickly *in vitro* and in such a manner as to form visible colonies within a reasonable time limit. Secondly, even if one succeeds in finding such media and cultural conditions resulting in a good formation of colonies, the finding hereof will be made easier if one was already able to observe a lesser growth under less ideal conditions. More sensitive methods might be found by observation of hanging drop cultures through the microscope. The disadvantage of these is however, that one does not know which is the most vital stage of the nodules and what one must therefore insert into these drops e.g. by means of a micromanipulator (hyphae, vesicles or bacteroids). Moreover the use of cloudy media, such as root- and soil suspensions, may cause difficulties during observation. The possibility of estimating growth by means of the increase of a property of the organism may also be born in mind. As only known property only the capacity to form nodules can be considered. In that case, however, one will have to know more about the factors which influence this nodulation

and, especially, one will have to examine whether a relation exists between the amount of endophyte in the inoculation matter and the number of nodules that can be formed and, in that case, what kind of a relation exists. In a subsequent publication in this series this item will be more closely studied.

SUMMARY

1. It is impossible to cultivate the endophyte from the root-nodules of the alder on the usual media *in vitro*.
2. Methods are described by which one can demonstrate with certainty whether certain organisms are capable of forming root-nodules in the alder.
3. The difficulties in connection with isolation are not caused by a dying off or removal of the endophyte during the required pre-treatments (disinfection with sublimate or bromium, removal of outer cell layers, suspension in water or nutrient media).
4. The endophyte is rather sensitive with regard to the influence of higher temperatures. During 1 hour at 60° C. the capacity to infect is completely lost.
5. Nodules which have remained visibly sterile after remaining for 6 weeks on a good nutrient medium, contain the endophyte still in its vital, virulent and efficient form.
6. Such nodules are very suitable as inoculation matter for work under sterile conditions.
7. With the aid of such nodules as inoculation matter elementary nitrogen fixation could be confirmed under pure conditions.

LITERATURE

- ALMON, L., 1933. Zentrbl. Bakt. etc. 2 Abt., 87: 289.
 BORM, L. 1931. Bot. Arch. 31: 441.
 BOTTOMLEY, W. B. 1912. Ann. Bot. 26: 111
 BOUWENS, H. 1943. Ant. v. Leeuwenhoek 9: 107.
 FERGUSON, T. P. and G. BOND, 1954. Ann. Botany N.S. 65, 175.
 GERRITSEN, F. C. 1935. Planta 23: 593.
 HILTNER, L. 1896. Landwirtsch. Versuchsstat. 46.
 HILTNER, L. & F. NOBBE. 1904. Naturw. Zeitschr. Land- u. Forstwirtschaft. 2: 336.
 JONGH, PH. DE. 1938. Verh. Kon. Akad. Wet. afd. Natuurk. 2 sect. 37: no. 6.
 KREBBER, O. 1932. Arch. Mikrobiol. 3: 588.
 KYLIN, A. 1950. Physiol. Plant. 3: 165.
 LAINE, T. & A. I. VIRTANEN. 1941. Die Stickstoffassimilation in BAMANN-MYRBÄCK: Methoden der Enzymforschung Tl. 3: 2728 Leipzig.
 PEKLO, J. 1910. Zentrbl. Bakt. etc. 2 Abt. 27: 451.
 PLOTHO, O. v. 1941. Arch. Mikrobiol. 12: 1.
 ROBERG, M. 1934. Ber. Deutsch. Bot. Ges. 52: 54.
 ROBERG, M. 1938. Jahrb. Wiss. Bot. 79: 472.
 ROBERG, M. 1938. Jahrb. Wiss. Bot. 86: 344.
 SCHAEDEER, R. 1933. Planta 19: 389.
 SHIBATA, K. 1902. Jahrb. Wiss. Bot. 37: 643.
 SHIBATA, K. & M. THAHARA. 1917. Bot. Mag. Tokyo. 31: 157.
 SPRATT, C. R. 1912. Ann. Bot. 26: 119.
 VIRTANEN, A. I. & S. SAASKAMOINEN. 1936. Biochem. Z. 284: 72.
 WAKSMAN, S. A. 1952. Soil Microbiology. New York.
 ZIEGENSPECK, H. 1929. Ber. Deutsch. Bot. Ges. 47: (50).

SYMBIOTIC NITROGEN FIXATION IN NON- LEGUMINOUS PLANTS

II. THE INFLUENCE OF THE INOCULATION DENSITY AND EXTERNAL FACTORS ON THE NODULATION OF ALNUS GLUTINOSA AND ITS IMPORTANCE TO OUR UNDERSTANDING OF THE MECHANISM OF THE INFECTION

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(Received Sept. 30th, 1954)

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I. INTRODUCTION

The formation of a root symbiosis is a complicated process, the details of which are only partially understood. We do possess many data on the influence of external factors on the nodulation of Leguminous plants, while many observations were described regarding the formation of mycorrhiza at different environmental circumstances. It is difficult however to get an impression of the way in which these external circumstances influence the formation of the symbiosis.

E.g. we do know that the nodulation of Leguminous plants is

inhibited by the presence of nitrogenous substances in the medium or by a decrease of the light intensity, while other factors like phosphate concentration or the presence of organic substances play a role as well. We refer to the compilations by FRED, BALDWIN and MCCOY (1932) and WILSON (1940). We don't know, however, why these factors influence the nodulation. The oldest hypothesis, that these external factors influence the concentration of sugars in the roots appeared to be too simplified. According to WILSON (1939) the C/N ratio might be the determining factor for the formation of nodules, each deviation in either direction from the ideal C/N ratio resulting in a decreased nodulation. Certainly this theory can describe all observations but an explanation of the observations is not possible as long as we do not know which of the carboniferous and nitrogenous substances are especially involved and why nodulation depends on their ratio.

One has to realize that internal factors are also of importance. It is well known that the different strains of *Rhizobium* not only differ as to their efficiency but as to their nodulating capacity as well: inefficient strains as a rule form more nodules though smaller ones than efficient strains. On the other hand NUTMAN (1949) draws attention to the genetic factors of the plants themselves which influence their nodulation. From different kinds of observations he draws the conclusion that the infection does not occur at random on the root surface but is only possible on certain preformed foci which are in some way related to the root initials (NUTMAN 1948, 1949, 1952, 1953).

As NUTMAN (1948) observed a correlation between the number of nodules formed and the initiation of lateral roots in a number of varieties of red clover it might be supposed that the genes which govern the formation of nodules primarily determine the potential number of foci. Other possibilities are that these genes might determine the concentration of food substances which are necessary for the development of nodules. Both the number of foci and the concentration of food substances may in their turn be influenced by the external circumstances.

Moreover the situation may be complicated by the possibility that the roots excrete chemotactic substances which attract the bacteria as the eventual production of such chemotactic substances might be influenced by the internal and external factors as well.

Therefore, when trying to survey the complex relationships which may exist between nodulation, internal and external factors we can classify the theoretically possible mechanisms of the influence of the external factors on the nodule production under the following four categories:

1. they might influence the plants either by changing the concentration of food substances (or their mutual ratio) or by changing the number of foci so that the roots become more or less liable to a subsequently occurring infection.
2. they might influence the infective capacity of the bacteria.

3. they might influence the process of the infection itself e.g. by influencing the production of chemotactic substances.
4. they might influence the growth of the infected foci into visible nodules.

It is no easy task to distinguish between these four categories of possibilities. One can only try to separate the first three categories which all bear directly upon the infection or the preparation of the infection from the fourth possibility which applies to the processes after an infection has occurred. This may be done by counting the number of nodules and not confining oneself to measuring the weight of the total quantity of nodules. However we are not justified in supposing that the number of nodules is not influenced at all by the subsequent growth after the infection has occurred. This is demonstrated by the extreme possibility that after a successful infection no growth at all might be possible.

As will be shown below an analysis of the relation between nodulation and inoculation density might be able to elucidate some of the difficulties described above. This relation, however, has never been analyzed in detail. The few publications dealing with the influence of the inoculation density and the number of nodules clearly demonstrate the cause of this gap in our knowledge (THORNTON 1929, BHADURI 1951). As the *Rhizobiums* are able to multiply in the vicinity of the roots the inoculation density can not be controlled: after some days the number of bacteria in the slightly inoculated experiments is as numerous as in the heavily inoculated ones.

It seemed justified to suppose that better results might be obtained with *Alnus glutinosa* as it has been shown that the endophyte which is responsible for the formation of the nodules in *Alnus* cannot multiply outside the host plant in the usual culture media (KREBBER 1943, BOUWENS 1943, QUISPÉL 1954). Even in root extracts, root homogenates and root exudates no growth was observed. If no multiplication of the endophyte in the vicinity of the roots is to be expected the inoculation density can be controlled and its effect on nodulation studied.

The main reason why I started this analysis, however, was of another kind. In the first publication of this series (QUISPÉL 1954) I stated that it would be very important to have a method of determining in a sensitive way the amount of the endophyte in a suspension or in an inoculated nutrient medium. Such a method might be based upon the relation between the amount of the endophyte in a solution and the number of nodules which can be formed by this solution.

Finally it was thought to be important to study the influence of external circumstances on nodulation in this non-leguminous plant and to compare the results with those obtained with Leguminous plants. The only investigation of this kind with *Alnus* (BJÖRKMAN 1942) already indicated that in *Alnus* the same relationships are found as in Leguminous plants.

II. METHODS

The methods used are the same as those described in the preceding article (QUISPEL 1954). The inoculation was always performed by using suspensions of crushed young nodules. In order to obtain an equal inoculation of all cultures it is advisable to filter the suspensions through glass wool. Variations in the inoculation density were obtained by dilutions of the original suspension. The nodulation was determined by the non-sterile method, each series consisting of 4 jars which contained 3 plants each. Only in the experiments on the influence of sugars the sterile method was used. In these experiments the sterile plants were inoculated with suspensions of crushed nodules which were "selected by incubation".

The definite count of the number of nodules was always performed 6-7 weeks after inoculation. Though the first nodules are already visible 3 weeks after inoculation most nodules are then so small that they can be easily overlooked, while after 6 weeks all nodules can be easily counted. A prolonged incubation did not increase the number of nodules. The nodules of *Alnus* have a branched form so that wherever an infection has occurred a branched group of nodules develops. If not stated otherwise such groups were counted as one nodule. In some experiments the total weight of the nodules was determined as well and from these determinations the average weight per nodule was calculated which gave an impression of the growth of the nodules.

The variation between the number of nodules of the individual plants of each series was always rather important. This was to be expected as I had to work with genetically heterogenous plants grown from seeds which were collected in the field. Therefore statistical analysis are necessary. In the tables I always mention the average number of nodules with the standard deviation of the average. As the number of experimental values in each series was rather small (12 or sometimes 11) its significance was estimated by using STUDENT's "t" analysis (FISHER 1948). As has been observed by NUTMAN (1952) in Leguminous plants here also the standard error increases with the average. The transformation on a logarithmic scale which is customary in such cases did not seem justified, as in this case as a result of the small values and the comparatively great deviations the resulting deviations from the normal frequency distribution would introduce still greater errors.

III. THE RELATION BETWEEN INOCULATION DENSITY AND NODULATION

a. THEORETICAL PART

Already from the first experiments which are not reproduced here it was evident that a definite relation could indeed be observed between inoculation density and nodulation. At slight inoculations the number of nodules increases with an increase of the inoculation

density till a maximum is reached where the number of nodules is independent of the inoculation density. This proves the supposition to be true that in *Alnus* the influence of the inoculation density could be better demonstrated than in Leguminous plants.

The relation between inoculation density and nodulation being experimentally analysable the question had to be asked what could be theoretically predicted about the nature of this relation. On closer examination it appears that the nature of this relation depends on the mechanisms of the infection. If we assume that the infection can occur anywhere on the root surface the relation between inoculation density and nodulation is rather simple. In this case we may assume that a doubling of the number of cells of the endophyte in the vicinity of the root will double the chance of the root surface being hit by cells of the endophyte. Consequently a linear relation between the inoculation density and the number of nodules has to be expected. This will be limited by the fact that the concentration of food substances e.g. sugars in the root does not allow for a further increase of the number of nodules. Moreover the possibility exists that the production of inhibiting substances by the developing nodules (NUTMAN 1952) limits the possible maximum number of nodules. The graphical curve showing the relation between the inoculation density and the number of nodules will more or less take the form of a typical "Blackman" curve consisting of two straight parts: one ascending part which shows the linear relation between inoculation density and nodulation and a horizontal part which shows the region where other factors are limiting a further increase of the number of nodules.

According to several investigators, especially NUTMAN, the infection does not occur at random on the root surface but is restricted to a limited number of certain preformed foci. If this is true the calculation of the probability of the occurrence of an infection must be based on the fact that a limited number of foci is surrounded by cells of the endophyte in the medium while now and then a focus is hit by a cell of the endophyte and infection is possible. It has been shown that in comparable cases (e.g. haemocytometer counts) the chance distribution is the distribution of Poisson: the number of foci which is never hit will be e^{-m} and the number of foci which is hit and infected will be $1 - e^{-m}$ where m is the mean number of hits per focus. As we may assume that the mean number of hits per focus is directly proportional to the number of cells of the endophyte surrounding the foci (x) we may write $m = c \cdot x$ in which c is a constant factor. If y is the number of nodules formed (= the number of foci which is hit) and A is the maximum number of nodules which is possible (= the total number of foci) we obtain the following relation:

$$y = A \cdot (1 - e^{-c \cdot x})$$

This is the well-known formula of Mitscherlich.

Of course the possibility exists that the infection is not a mere game of chance but that chemotactic substances play a role. These substances may be excreted either by the whole root surface or by

the foci themselves. If the first is true this excretion will result in a greater concentration of the endophyte in the rhizosphere of the roots but in this concentrated rhizosphere the infection will happen in the same way as described above so that the same theoretical considerations hold true. If the chemotactic substances are excreted by the foci each focus will be surrounded by a concentration gradient of these substances. An infection will occur always whenever a cell of the endophyte enters into the attractive zone around a focus and again the Mitscherlich relation will be found. It is unlikely that in our well-shaken experiments the attractive influence of the excreting foci would be exerted over a long distance. If, however, this should occur all cells of the endophyte in the vicinity of the roots will be directly attracted into the foci. Now an influence of the inoculation density will only be observed as long as there are too few cells of the endophyte available to infect all foci; in this case the number of nodules will be identical with the number of cells of the endophyte and the relation between inoculation density and nodulation will be of the "Blackman" type.

Therefore, depending on the fact whether the infection occurs everywhere on the root surface or is restricted to preformed foci and in the latter case whether the infection is a mere game of chance or is dominated by the activity of chemotactic influences the relation between inoculation density and nodulation will be of the "Blackman" or the "Mitscherlich" type.

b. EXPERIMENTAL PART

It is no easy task to distinguish experimentally between these two possibilities as the variation of the observations is rather large while the difference between the two theoretical curves is rather small. The difference between the "Blackman" curve with its two straight parts and the more smoothly curved "Mitscherlich" curve is most evident at the point where in the "Blackman" curve the transition is found between the ascending and the horizontal part of the curve. Attention has to be focussed on observations in this region and it has to be calculated whether the number of nodules which is observed here is consistent with either the ascending or the horizontal part of the "Blackman" curve which is calculated from the other observations. On the other hand one has to examine whether the observations are consistent with a "Mitscherlich" relation.

In table 1 and fig. 1 the results are summarized of an experiment in which the relation between inoculation density and nodulation was determined by inoculating a number of jar-cultures of *Alnus* with dilutions from the same suspension of nodules. In fig. 1 the best fitting "Mitscherlich" curve is drawn and it is clear that no significant departures from the theoretical values occur. However, this experiment is not yet conclusive. It is evident that the number of nodules obtained with the inoculation "75 mgr" is far below the value which could be calculated by extrapolation of the best fitting straight line through the values obtained with "0.1", "1", "10"

TABLE 1

Relation between inoculation density and nodule formation

Inoculations were performed with dilutions of a suspension of crushed nodules in water, the inoculation density is expressed as mgr fresh-weight of nodule material inoculated per jar. Each jar contained 2-3 plants, each series consisted of 4 jars. The inoculation was performed 4 II 53, the number of nodules counted 18 III 53

Inoculation density	Number of plants	Number of nodules (average with stand. dev. average)
300	12	29.9 ± 5.8
150	12	32.4 ± 7.4
75	11	20.9 ± 2.9
25	12	19.3 ± 4.8
10	11	7.3 ± 1.8
1	12	2.3 ± 0.7
0.1	12	0.2 ± 0.1

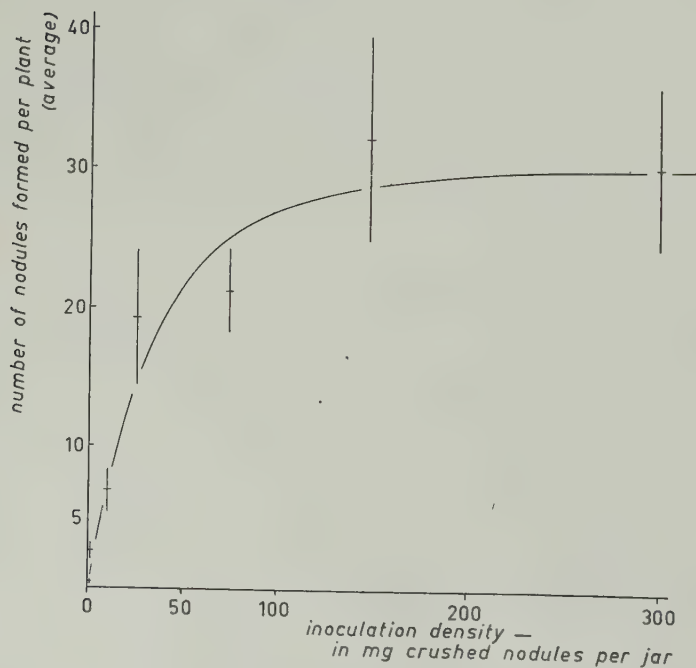


Fig. 1. Relation between inoculation density and nodulation according to the experimental results of table 1

and "25 mgr.". However, at inoculation with "75 mgr." the maximum number of nodules might have been reached already as the difference with the mean of the number of nodules obtained with the inoculations "150" and "300 mgr." is not yet significant. ($n = 33$, $t = 1.427$, $P = 0.1-0.2\%$).

In table 2 and fig. 2 the results are shown of another experiment

TABLE 2

Relation between inoculation density and nodule formation

Inoculations were performed with dilutions of a suspension of crushed nodules in water, the inoculation density is expressed as mgr fresh-weight of nodule material inoculated per jar. Each jar contained 2-3 plants, each series consisted of 4 jars. The inoculation was performed 25 VIII 52, the nodules were counted 8 X 52

Inoculation density	Number of plants	Number of nodules (average with stand. dev. average)
100	10	61.3 $\pm$ 11.0
10	10	24.8 $\pm$ 4.5
1	12	6.6 $\pm$ 1.6
0.1	11	0.5 $\pm$ 0.2

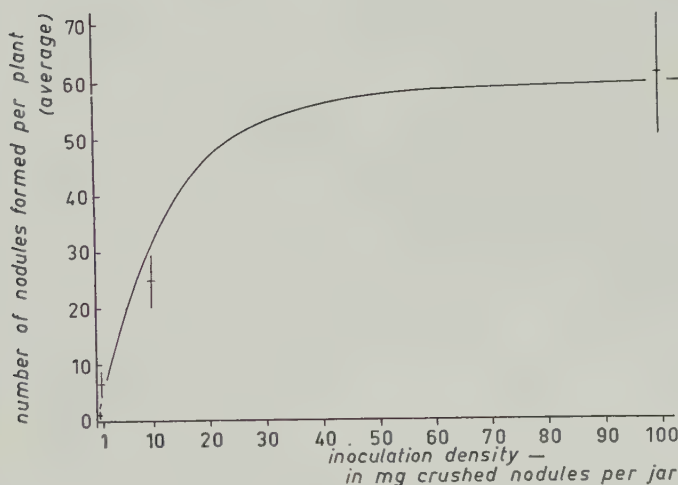


Fig. 2. Relation between inoculation density and nodulation according to the experimental results of table 2

which is less illustrative in consequence of a smaller number of observations. The number of nodules which is obtained with the inoculation "10", however, significantly differs from the "Blackman" curve which can be constructed through the other observations. If we extrapolate the best fitting straight line through the values obtained with the inoculations "0.1" and "1" to the inoculation "10" we obtain a value of 65.6 ± 4.1 . This value is of the same order as the value obtained with the inoculation "100" and has to be regarded as the maximum number of nodules which is possible under the conditions of this experiment. The observed number of nodules at incubation "10", however, significantly differs from this maximum ($n = 18$, $t = 2.93$, $P = 0.01$).

As in both experiments the deviations are observed in the region

and in the direction where they have to be expected and as in both experiments a "Mitscherlich" curve may be constructed which shows no significant departures from the observed values the choice between the "Blackman" and the "Mitscherlich" type of curve must be made in favour of the "Mitscherlich" curve. In none of all other experiments, which are not reproduced here, a significant departure from a "Mitscherlich" curve was observed.

I have to mention one possible source of error. As we can only inoculate with dilutions of suspensions of crushed nodules the possibility exists that the results are influenced by the presence of eventual inhibiting substances from the nodules of the inoculums. As the concentration of these substances will be greater in the heavier inoculations the number of nodules produced by these heavier inoculations might be decreased. This possible source of error can only be excluded with certainty when we possess *in vitro* cultures of the endophyte. However, it is unlikely that our results were seriously influenced by the presence of inhibiting substances. In one experiment some jars with *Alnus* plants were inoculated with a suspension of crushed nodules while another set of jars was inoculated with the same suspension after removal of all soluble substances by "Seitz" filtration. No significant difference between the number of nodules in these two sets of jars was observed, so that at least no indication was obtained for the occurrence of soluble inhibitors in concentrations as might influence experiments of this type.

Of course the results obtained do not prove that the "Mitscherlich" formula is indeed the exact formulation of the relation between the inoculation density and nodulation. It only proves that the "Mitscherlich" formula is not contradicted by the experimental results while the "Blackman" relation gives more or less significant differences between the theoretical and observed values.

The conclusion may be that the experimental results are in accordance with the theory that the infection occurs at preformed foci. Of course they cannot give any information as to the nature of these foci (root initials, polyploid cells?) nor can they inform us about an eventual activity of chemotactic substances during the infection.

IV. THE INFLUENCE OF SOME ENVIRONMENTAL FACTORS ON THE NODULATION

It may be expected that, as in Leguminous plants, the number of nodules of *Alnus* will be influenced by certain environmental factors. In the experiments to be described below the influence of some of these factors will be shown. In order to eliminate the influence of the inoculation density these experiments were inoculated with such heavy inoculations that the maximum nodulation was approximated. Only in the experiments on the influence of sugars the amount of "sterile" nodules was not large enough to reach this maximum.

a. THE INORGANIC COMPOSITION OF THE MEDIUM

In the usual experiments the plants are cultivated on a normal Hoagland solution which, at the time of the inoculation, is renewed by a nitrogen-free solution. In the experiments in which the influence of the inorganic composition of the medium on the nodulation was examined the composition of the nitrogen-free solution was changed by the addition of nitrogenous substances (ammonium sulfate of potassium nitrate) or by varying the concentration of fosfate. Care was taken that the total concentration of potassium and calcium remained the same (by addition or omission of their chlorides) and that in all solutions the pH remained at $\text{pH} = 5.4$. I want to stress the fact that the variations in the composition of the medium were only present from the moment inoculation takes place. Contrary to other experiments the nodules of each infection group were counted individually so that the number of nodules is 3–4 times greater as compared with other experiments where only groups of nodules were counted together. The results are given in table 3.

TABLE 3

The influence of the inorganic composition of the medium on nodule formation

Inoculations were performed with a suspension of crushed nodules corresponding with 30 mgr fresh-weight per jar. Each jar contained 3 plants, each series consisted of 4 jars. The inoculation was performed 1 V 51, the number of nodules was counted 18 VI 51

Composition of the medium in milliaeq. N or P			Number of nodules	Weight of the nodules	
NH ₄	NO ₃	PO ₄		Per plant	Per nodule
0	0	0.25	106.5 $\pm$ 15.5	56.2	0.53
0	0.375	0.25	176.8 $\pm$ 18.8	84.0	0.49
0.375	0	0.25	9.1 $\pm$ 3.4	29.2	3.21
0	3.75	0.25	0.4 $\pm$ 0.3	0.7	1.6
3.75	0	0.25	0.3 $\pm$ 0.3	0.3	1.0
0	0.375	0	14.4 $\pm$ 2.9	3.5	0.25
0.375	0	0	0	0	0
0	0	0	18.7 $\pm$ 7.1	18.0	0.95
0	0	2.5	98.7 $\pm$ 10.9	47.7	0.50

The results are in accordance with what could be expected on the ground of the results which have been obtained with Leguminous plants and the few experiments performed with *Alnus* by BJÖRKMAN. The presence of bound nitrogen has a definite inhibiting effect on the formation of root nodules. This effect is especially related to the infection itself and not so much to the subsequent growth of the nodules. This may be concluded from the observation that in the presence of 0.375 m.aeq.NH₄ the few nodules which still develop are much heavier than the many small nodules which develop in nitrogen-

free solutions. Very small additions of bound nitrogen may result in a small but significant increase of the number of nodules. The difference between NO_3 and NH_4 additions may be caused by secondary effects. The influence of fosfate is very much in evidence: when fosfate is absent the number of nodules is definitely decreased. This is the more remarkable as the plants received enough PO_4 till the moment of the inoculation while the plants did not show any symptoms of fosfate deficiency till the end of the cultivation period. Higher gifts of PO_4 as were present in the usual Hoagland solution had no effect on the nodulation.

b. THE LIGHT CONDITIONS OF THE PLANT

The influence of the light conditions was examined by changing the light intensity and by shortening the day length. The light intensity was changed by putting some plants underneath a box of filter paper which decreased the light intensity to $\pm 1/5$, while the other plants remained in the full day light of the summer months. The day length was shortened by putting some plants from 17–9 o'clock in absolute darkness. In order to test the possibility that the C/N ratio in the roots might be the determining factor these experiments were performed with plants grown on the normal nitrogen-free solution and plants grown on solutions with the small amount of KNO_3 which had proved to be beneficial in the preceding experiment. All variations in light intensity, day length and nutrient solution were present from the moment inoculation takes place. The results are shown in table 4.

TABLE 4

The influence of the light-conditions on nodule formation

Inoculations were performed with a suspension of crushed nodules corresponding with 30 mgr fresh-weight per jar. Each jar contained 3 plants, each series consisted of 4 jars. The inoculation was performed 15 VI 51, the nodules were counted 8 VIII 51

Light condition		N-content of the medium in m. aeq. NO_3	Number of nodules	Weight of the nodules	
Day-length	Intensity			Per plant	Per nodule
normal	normal	0	58.3 ± 14.4	88.3	1.5
normal	normal	0.375	78.8 ± 14.3	125.5	1.6
short	normal	0	50.2 ± 7.0	38.1	0.8
short	normal	0.375	59.2 ± 10.1	59.2	0.9
normal	low	0	32.8 ± 8.5	20.7	0.6
normal	low	0.375	25.0 ± 5.4	22.4	0.9

The differences obtained in this experiment are for the most part not significant. However, as they agree with the results obtained in

other experiments, it is justified to draw the following conclusions. Both the lowering of the light intensity and the shortening of the day length result in a decreased number of nodules. It cannot be decided whether the unfavourable effect of short days is caused by the smaller total amount of light energy per day or by the day length as such. The largest decrease occurs when small amounts of bound nitrogen are present. This might point to the possibility that in *Alnus* as well the C/N ratio is the determining factor in nodulation.

c. THE PRESENCE OF ORGANIC SUBSTANCES

In all hypothesis which try to explain the influence of external factors on nodulation attention is paid to the presence of organic food substances, especially of sugars, in the roots, either as such or in relation to the nitrogenous substances. It is generally expected that the addition of sugars in the culture solution might favour the production of nodules and the few experiments with Leguminous plants confirm this expectation. As such experiments must be performed under sterile conditions, at least of the roots, they are rather scarce.

The experiments regarding the influence of sugars on nodulation of *Alnus* were performed with plants with sterile roots and inoculated with "sterile" nodules. In consequence the experimental series could not be as great as in the other experiments. In table 5 the results of three small experiments, performed in the summer and in the winter months, are collected. The addition of glucose was performed at the moment of the inoculation when the plants were transferred to the nitrogen-free solutions while the level of glucose was kept constant by the weekly renewals.

TABLE 5

The influence of glucose on nodule formation

Inoculations were performed with suspensions of crushed "sterile" nodules in cultures of *Alnus* with sterile root systems. Experiment A was inoculated 8 VIII 51 and counted 11 IX 51, exp. B was inoculated 22 I 52 and counted 11 III 52 and experiment C was inoculated 19 VI 52 and counted 11 VIII 52. The figures represent the counts of nodules in the individual cultures.

Concentration of glucose in %	Number of nodules in the cultures of		
	exp. A	exp. B	exp. C
0	0-4-15-3	2-1-4-0	2-9-14-7
0.1	0-4-0-5	0-0-0-2-0-1	
1	0-0-0-0	0-0-0-0-0-0-0	0-0-0

There can be no doubt that the presence of glucose has an inhibiting effect on nodulation. This was observed in experiments which were performed during the good light conditions of the summer and during the smaller light intensities of the winter months. This is

contrary to what might be expected and which indeed had been found with Leguminous plants. The question arises whether this inhibiting effect could not have been caused by the presence of e.g. reductones which were produced during the heat sterilization of the glucose. Therefore the experiment was repeated but now the glucose was sterilized separately by filtration through a Jena G5 filter. The results of this experiment are given in table 6.

With this experiment the inhibiting effect of the glucose on nodulation is not as large as it was in the preceding experiments.

TABLE 6

The influence of glucose (sterilized by filtration) on nodule formation

Inoculations were performed with a suspension of crushed "sterile" nodules in cultures of *Alnus* with sterile root systems. The inoculation was performed 31 VII 53, the nodules were counted 17 IX 53

Concentration of glucose in %	Number of plants	Number of nodules per plant (average with stand. dev. average)
0	9	3.3 $\pm$ 0.6
0.1	7	1.1 $\pm$ 0.5
1	9	1.2 $\pm$ 0.3

This is an indication that indeed substances, which arise from the glucose during the heat sterilization, were responsible for a part of the inhibiting effect. However, even in this experiment, the inhibiting effect is significant for both concentrations of the glucose ($n = 14$, $t = 2.69$, $P = 0.01-0.02$ and $n = 16$, $t = 3.18$, $P = 0.01$ respectively).

Finally it may be mentioned that in another experiment sucrose appeared to inhibit nodulation in the same way. So we have to conclude that the number of nodules is decreased by the presence of sugars in the culture solution. Further experiments are necessary before we can give an explanation for this remarkable and unexpected effect.

d. CONCLUSION

The conclusion drawn from this part of the investigation can be that the presence of bound inorganic nitrogen, fosfate and the light conditions exert the same influence on the nodulation of *Alnus* as they exert on Leguminous plants. The necessity of good light conditions, the inhibiting effect of ammonium salts and nitrates as well as the promoting effect of these salts in very small concentrations agree with the idea that the number of nodules depends on the ratio between carboniferous and nitrogenous substances in the roots. However, the inhibiting effect of glucose and sucrose is not in accordance with this idea. A final explanation can only be given when we know which carboniferous and nitrogenous substances are especially concerned and at which moment in the chain of processes leading to nodulation they exert their influence.

V. THE INFLUENCE OF THE INOCULATION DENSITY ON NODULATION IN ITS RELATION TO THE INFLUENCE OF EXTERNAL FACTORS

a. THEORETICAL PART

It is very difficult to ascertain what is the crucial moment in the nodulation process where the external factors exert their effect on the formation of the nodules. In the general introduction the possibilities could be divided into four categories. If any of these four possibilities would be realized certain consequences regarding the relation between inoculation density and the number of nodules and the way in which this relation itself is affected by the influence of the external factors would result. We shall now consider the consequences with respect to all these possibilities. ¶¶

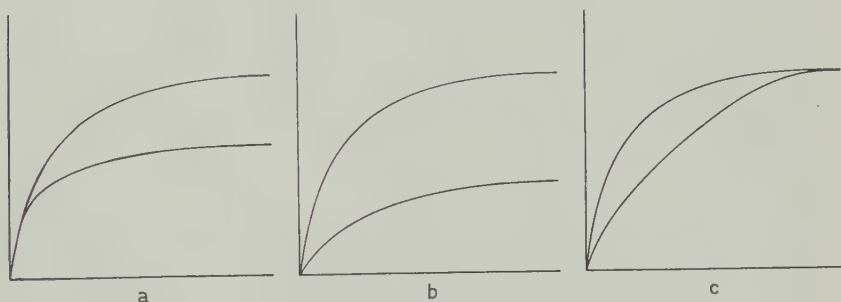


Fig. 3. Theoretical relations between inoculation density and nodulation at different external circumstances (explanation in text)

Possibility 1. a. *The external factors influence the plant by changing the concentration of certain food substances in the roots so that they become more or less liable to the occurrence of an infection.*

If indeed the concentration of food substances is the crucial point we may assume that the higher the number of nodules the sooner the lack of food substances will be evident. After heavy inoculations the lack of certain food substances will sooner be of importance than after slight inoculations when the small amount of food substances is still sufficient for the small number of nodules. The influence of the external factors which govern the amount of food substances will be especially apparent at the heavier inoculations while at the slightest inoculations no effect will be observed. The curves which show the relation between inoculation density and nodulation will change as is schematically shown in fig. 3a. If we compare the Mitscherlich formulae at different external circumstances the values of both A and c will be different. At the most unfavourable circumstances definite departures from a Mitscherlich relation will be found.

When considering the possibility of the formation of nodules being limited by mutual excretion of inhibiting substances by nodules while the formation of these substances is influenced by external factors we

arrive at the same results as the effect of the amount of these inhibitors will be most manifest if the nodules are close together (after heavy inoculations).

Possibility 1. *b. The external factors influence the plant by changing the number of foci which can be infected by the endophyte.*

The consequence of this possibility is the following: The total number of foci is identical with the maximum number of nodules, so that after heavy inoculations when the maximum number of nodules is approximated the number of nodules will depend on the external factors which influence the number of foci. After slight inoculations the chance of the cells of the endophyte to hit a focus will be decreased if the number of foci is decreased, so that at this stage the number of nodules will depend on the external circumstances as well. The actual mechanism of the infection, however, will remain unaffected. The curves of the relation between inoculation density and nodulation will change with the external factors as is schematically illustrated in fig. 3*b*. In the Mitscherlich formula A will change with the external factors, but c will remain the same.

Possibility 2. *The external factors influence the infective capacity of the bacteria.*

Though the presence of nitrogenous or organic substances in the medium of the roots might possibly influence some properties of the endophyte so that their infective power is affected it is absolutely inconceivable that e.g. the light conditions influence the cells of the endophyte in the surroundings of the roots in a direct way. This possibility can never be considered to be the sole or even the main cause of the influence of these external factors.

Possibility 3. *The external factors influence the process of the infection itself e.g. by influencing the formation of eventual chemotactic substances.*

If the process of the infection has to be considered as a mere game of chance it is unlikely that it is influenced by the external factors observed. If, however, chemotactic substances play a role, the external factors might influence the excretion of such substances. This will affect the chance of the foci being hit and infected, but the total amount of possible infections will remain the same. The influence of the external factors would, in this case, occur according to the scheme of fig. 3*c*. In the Mitscherlich formula c will be influenced while A remains unchanged.

Possibility 4. *The external factors influence the growth of the infected foci into visible nodules.*

As in all our experiments the number of nodules was primarily concerned this possibility can only have been of secondary importance. In those experiments where both the number and the weight of the nodules was measured it was observed that the influence on the number of nodules sometimes far exceeded that on the weight of

the nodules. Of course we must reckon with the theoretical possibility that in some experiments the infection had succeeded but could not be observed as none of the infected foci developed into nodules. Certainly the growth of the nodules will depend on external factors but the design of our experiments was such that the main attention was focussed on the influence of external factors on the events prior to and during the infection itself.

So these theoretical considerations offer a possibility of experimentally distinguishing the way in which the formation of nodules is influenced by external factors. As the possibilities 2 and 4 can be excluded we can distinguish between the possibilities 1a, 1b and 3 by determining the relations between inoculation density and nodulation at varying external circumstances. If the Mitscherlich formula is the best description of this relation we must determine whether the values of A , c or of both are influenced by the external factors.

b. EXPERIMENTAL PART

A consideration of the experiments of chapter III already shows that those possibilities according to which the factor A remains constant are not in accordance with the facts. E.g. in the experiments of table 3 and 4 the inoculation has certainly been so heavy that the number of nodules can be considered to approximate the maximum value of A . Nevertheless great differences due to the external influences are observed. So the possibilities 3 (influence on the production of eventual chemotactic substances) can be excluded. The question remains whether the value of c remains constant or is influenced as well.

A practical difficulty here arises. It might be justified to compare the values of c obtained from different experiments if in all these experiments the inoculation was performed with absolutely known quantities of endophyte. However, as we don't possess cultures of the endophyte we have to inoculate with suspensions of crushed nodules of which the absolute content of endophyte is unknown. Therefore it is only possible to compare experiments which are inoculated with dilutions of the same nodule suspension, while it is impossible to compare experiments which are inoculated with dilutions from different nodule suspensions. In consequence: if we try to determine the value of c in the Mitscherlich formula we cannot obtain absolute values. We can only obtain values of the product $c \cdot x$, but we cannot determine which part of it belongs to x and what part of it belongs to c . If we want to examine the effect of external factors on c we must be sure that x is the same in all experiments. In consequence we can only compare experiments which were inoculated at the same moment but at different external circumstances with dilutions from the same suspension of crushed nodules.

The first experiments which allowed for such a comparison did not answer the question in a convincing way. Though there was no reason to doubt the constancy of c the variation of the observations

was too great to establish this constancy without any doubt. I therefore performed a new experiment with more plants per series (24) so that the influence of the variation was restricted. Three inoculation densities were compared. Unfortunately these densities were obtained by dilutions of a suspension of nodules which appeared to be only slightly active, so that in the heaviest inoculation the maximum number of nodules was not yet obtained and the value of A had to be calculated from the other values. Half of the plants remained in the normal light conditions of summer (the experiment was inoculated 26 V 53), the other half of the plants was cultivated at lower light intensities by putting the plants underneath a box of filter-paper which reduced the light intensity to $\pm 1/5$. The results are shown in table 7 and fig. 4.

TABLE 7

The influence of inoculation density on nodule formation at different light conditions

Inoculations were performed with dilutions of the same suspension of crushed nodules in water, the inoculation density is expressed as mgr fresh weight of nodule material inoculated per jar. Each jar contained 3 plants, each series consisted of 8 jars. The figures between brackets are calculated from the Mitscherlich formulae (see text). The inoculation was performed 26 V 53, the number of nodules was counted 16 VII 53

Inoculation density	Number of nodules (average with stand dev. of average)	
	Normal light intensity	reduced light intensity
300	25.3 $\pm$ 1.8 (23.7)	4.1 $\pm$ 0.6 (4.2)
50	4.5 $\pm$ 0.6 (5.6)	0.9 $\pm$ 0.2 (1.0)
10	1.2 $\pm$ 0.2 (1.2)	0.5 $\pm$ 0.1 (0.2)

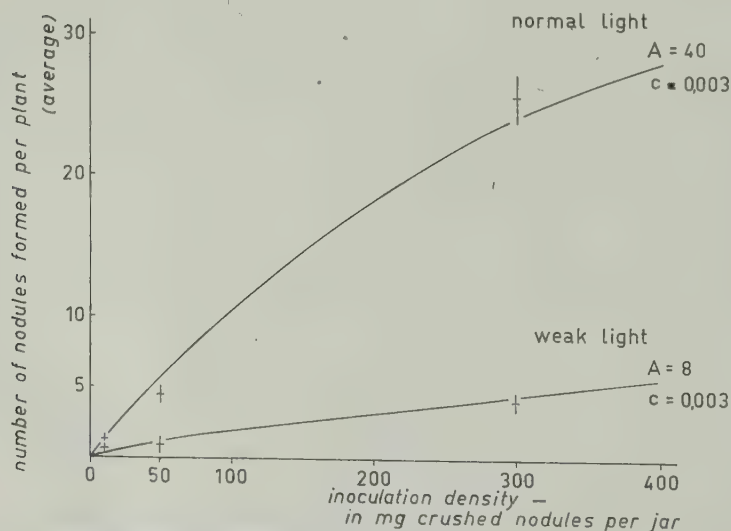


Fig. 4. Relation between inoculation density and nodulation at different light conditions according to the experimental results of table 7

In the first place it is again evident that the law of limiting factors in its simplest shape does not hold true. The influence of the light intensity is already manifest at the slightest inoculations. Again this experiment gives additional and convincing evidence that the relation between inoculation density and nodulation cannot be described by a simple „Blackman” relation as the influence of the external factor already is manifest in a region where the inoculation density is a limiting factor. If we calculate the best fitting constants of the Mitscherlich formula we obtain for the high light intensity $A = 40$ and $c = 0.003$, for the low light intensity $A = 7$ and $c = 0.003$. The theoretical values for the three inoculation densities which are calculated from these constants are inserted between brackets in table 7. The agreement between the theoretical and experimental values requires no further comment. There is no reason to assume that the value of c is influenced by the light conditions. Only the value of A is changed. Of all possibilities mentioned in the beginning of this chapter only the possibility 1 a is in accordance with the facts. The experimental results may be entirely explained by assuming that the light conditions alter the number of foci on which the infection has to occur, the infection itself being a mere game of chance. Though the possibility is not excluded that chemotactic substances play a role, the production of these substances is certainly not changed in such a way that it is experimentally demonstrable.

VI. DISCUSSION

A theoretical discussion of the relation between inoculation density and nodule formation shows that the nature of this relation is different whether we base our considerations on the assumption that the infection may occur at random everywhere on the root surface or on the assumption that the infection can only occur in a limited number of preformed foci. In the first case a linear relation between inoculation density and the number of nodules has to be expected which will only be limited at heavier inoculations when food substances become limiting factors or when the mutual secretion of inhibiting substances limits a further increase of the number of nodules. In the second case an application of the theory of chances shows that the relation between the number of nodules y and the number of cells of the endophyte in the medium x can be described by a Mitscherlich formula $y = A (1 - e^{-c \cdot x})$ where A is the number of foci = the maximum number of nodules and c is a constant factor. While the relation between inoculation density and nodulation cannot be studied in Leguminous plants as the number of *Rhizobium* cannot be effectively controlled as they multiply in the medium around the roots, this study is possible with plants of *Alnus* as here the endophyte cannot develop in vitro. The experimental results obtained by inoculating a number of *Alnus* plants with different dilutions from a suspension of crushed nodules shows that the assumption of a Mitscherlich relation is better in accordance with the facts. This may

be used as another argument for the theory of NUTMAN, that the infection can only occur in a limited number of preformed foci. Of course the inoculation density is not the only factor which influences the formation of nodules as the number of nodules is influenced by a number of external factors. Nitrates and ammonium salts inhibit the nodulation completely though in very small concentrations a weak stimulation is observed. Moreover the formation of nodules depends on the presence of fosfate and good light conditions. There are a few indications that, as in Leguminous plants, it is especially the C/N relationship in the roots which governs the formation of nodules. The inhibiting effect of added sugars is unexpected and, for the time being, unexplained.

If the inoculation density and the light intensity are simultaneously varied it appears that the relation between the inoculation density and the number of nodules at two light intensities can be described by the same Mitscherlich formula with the same value for c but different values for A . This is in accordance with the expectation when we assume that the light intensity only influences the formation of nodules by influencing the number of preformed foci on which the infection can only occur. The infection itself is determined by the chance that a focus is hit by a cell of the endophyte. If the influence of changed external conditions depends mainly on the presence of available food substances or on the formation of eventual chemotactic substances the value of c might be expected to change as well. Though of course we have to be aware for premature generalizations there are reasons to suppose that the same will be true with regard to the influence of nitrogenous substances as there are definite relations between the effect of light and the effect of nitrogenous substances. Moreover it has been observed that after addition of nitrogenous substances a few nodules develop, the weight of these nodules exceeding the weight of the nodules on nitrogen free media, so that the decrease of eventual food substances could never account for the decrease of the number of nodules.

On the ground of the described experiments we can form the following notion of the process of the infection. The endophyte cannot enter the roots everywhere on their surface but can only enter the roots on a limited number of preformed foci. The number of these foci is influenced by genetical factors as well as by some external conditions like light conditions and most probably also by the presence of nitrogenous substances, fosfate and so on. The infection itself is a mere game of chance, the number of foci which is infected depending on the inoculation density as may be expected according to the chance distribution of Poisson. It cannot be concluded from our experiments whether chemotactic substances play a role either by concentrating the endophyte in the rhizosphere or by attracting the endophyte within a short distance of the foci. Once the focus is infected the growth of the nodule will certainly depend on the available food substances.

I wish to stress the fact that this notion regarding the infection process

cannot pretend to be more than a hypothesis most in accordance with the experimental results. The truth of this hypothesis must be verified by other experiments. It is impossible to say anything more about the nature of the postulated foci. NUTMAN supposed that a relation exists between such foci and the root initials. Indeed the formation of root initials as well is influenced by light conditions while it is a common observation that nitrogen starvation results in extensive root formation. Certainly, however, these foci cannot be identified with the root initials as in the presence of normal amounts of nitrates a normal root development is observed while no nodules can be formed. Perhaps the foci are identical with the few polyploid cells in the neighbourhood of the initials (WIPF and COOPER 1940).

Finally I have to mention a practical consequence of the results which is very important for the further study of the root nodule symbiosis of non-leguminous plants. The definite relation between inoculation density and nodulation and the simple way in which this relation is influenced by the external factors provides a possibility of determining in a semi-quantitative way the amount of endophyte in unknown suspensions and inoculated culture media. By using this method it should be possible to measure an eventual growth of the endophyte in such media in a fairly accurate way. The practical execution of this method and its results will be described in the next article of this series.

SUMMARY

1. The relation between inoculation density and the number of nodules in watercultures of *Alnus glutinosa* is in accordance with the hypothesis that the infection occurs through a chance contact of the endophyte with a limited number of preformed foci.
2. The influence of some external factors on the formation of nodules is principally the same as has been observed in Leguminous plants. Nitrates and ammonium salts inhibit nodule formation while fosfates and good light conditions are necessary.
3. The formation of nodules is inhibited by the presence of sugars in the nutrient solution.
4. A further analysis of the mutual effects of inoculation density and light intensity on the formation of nodules suggests that the light intensity influences the number of foci.

REFERENCES

- BHADURY, S. N. 1951. *Ann. Botany* 15: 209.
BOUWENS, H. 1943. *Anth. v. Leeuwenhoek* 9: 107.
FISHER, R. A. 1948. *Statistical methods for research workers*. 10th ed. Edinburgh-London.
FRED, E. B., I. L. BALDWIN and E. MCCOY. 1932. *Root nodules and leguminous plants*. Madison.
KREBBER, O. 1932. *Arch. Mikrobiol.* 3: 588.
NUTMAN, P. S. 1948. *Ann. Botany N.S.* 12: 8.
NUTMAN, P. S. 1949. *Heredity* 3: 263.
NUTMAN, P. S. 0000. *Ann. Botany N.S.* 13: 261.
NUTMAN, P. S. 1952. *Ann. Botany N.S.* 16: 79.
NUTMAN, P. S. 1953. *Ann. Botany N.S.* 17: 95.
QUISPÉL, A. 1954. *Acta Bot. Neerl.* 3: 495.
THORNTON, H. G. 1929. *Jour. Agric. Sci. (England)* 19: 373.
WILSON, P. W. 1940. *The biochemistry of symbiotic nitrogen fixation*. Madison.
WILSON, P. W. and E. B. FRED. 1939. *Jour. Amer. Soc. Agron.* 31: 497.
WIPF, L. and D. C. COOPER. 1940. *Amer. Journ. Botany* 27: 821.

IMPROVED YIELDS IN ALGAL MASS CULTURES

BY

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126th Communication of this laboratory, 42nd Communication on Photosynthesis

(Received Oct. 9th, 1954)

During the past few years considerable attention has been given to economic possibilities, offered by large scale cultures of green algae. Most of the earlier work (uptil 1952) in this field was surveyed in a recent publication of the Carnegie Institution of Washington [1]. In brief it could be said, that the many promising aspects of algae cultures, as compared to conventional methods of producing food, are to a certain extent balanced out by the higher cost and investments needed. In this respect, the yields obtainable per unit area are of primary importance—at least as long as the bulk material is more important than special products are.

The best yields actually observed by a number of workers ranged from 8–12 grams per m² exposed area per day [1] pp. 60, 113, 134,¹ 147,¹ 175, 271, 280. Optimal yields obtained earlier in our group during short periods amounted to 12.5 gr/m² day [1] p. 60. [2] This represented the fixation of about 4 % of incident solar radiation, in the range of 400–700 mμ. It was felt, that the main yield limiting factor was that the intensity of bright sunlight could not be used efficiently by the algae studied.

We hoped to improve outdoor yields by using strains of algae with higher photosynthetic capacity in utilizing strong light. Therefore a number of strains collected was tested under conditions of high light intensity at various temperatures. By prolonged exposure to such conditions it was tried to further adapt algal material.

During the summer of 1953 the growth yields of a number of apparently favourable strains were studied in outdoor cultures.

I. SELECTION OF ALGAL STRAINS

METHOD OF CULTIVATION

Glass-walled water tanks (50 × 25 × 25 cm) were arranged in such a way, that their temperatures could be kept at any desired

¹ The figures given by the authors were calculated on a basis of the cross-sectional area of the cylindrical culturing devices used. A correction with $2/\pi$ cf. p. 000 probably has to be applied in these cases.

value between 15 and 50° C. By using two regulators in each bath and switching from one to the other every 12 hours, a choice of "day" and "night" temperatures was available. In three tanks the "day" temperatures were set at 40, 30 and 20° C. respectively, while the corresponding "night" temperatures were 25, 20 and 15° C. During the "day" periods the algae were illuminated with 500 Watt incandescent lamps with internal reflectors (Philips Altrilux). Each lamp yielded an intensity of ca. 0.25 cal/cm² min. of photosynthizable radiation ($\lambda < 700 \text{ m}\mu$), corresponding to about 40 klux over an area of ca. 200 cm². This area was sufficient to illuminate 16 test tubes, each containing 10 ml of algal culture or a smaller number of larger vessels. Per tank 4 of these lamps could be used. The cultures were grown in inorganic nutrient solution (cf. 1, pg. 59), containing 0.05 m KNO₃ and were continuously flushed with air, enriched with 5 % CO₂.

In order to have individual cells exposed to as high an intensity as available, care was taken to keep the cultures thin. The test tubes were often—the fast growing ones daily—diluted by simply discarding the contents and adding fresh sterilized medium. The frequent handling of the cultures and their continuous aeration exposed these experiments to hazards of contamination. First of all, however, our main aim was a practical one: to obtain fast growing strains under the conditions chosen. The results proved quite reproducible: repetition of exposures starting afresh from the pure cultures kept on agar slants in a light cabinet, usually yielded identical results.

METHODS USED FOR STUDYING GROWTH RATES

For a study of algae with respect to usefulness for massculture application, measurement of growth in terms of dry weight (preferably expressed per unit irradiated surface or still better per unit absorbed light) may be considered as most straight forward, since it yields the sumtotal of all intricate processes involved.

A semi-quantitative judgement of the growth rates in thin sterile cultures was obtained by the following procedure:

From each culture to be tested we equally inoculated a number of test tubes provided with culture medium. Two test tubes were placed in each of the tanks, and a parallel sample of the inoculum was used for estimating its dry weight.

After the samples had grown dense enough to absorb about 80 % of the incident light, their dry weight was estimated. From initial and final dry weights (W_i and W_f) and the time of exposure to the light (t) a "generation time" τ was computed according to:

$$\tau = -0.3 t \log W_i/W_f \quad (1)$$

The procedure followed was a little arbitrary since the size of the inoculum and the moment of harvesting were not exactly defined. Not too much weight therefore can be given to the absolute time values found and only data obtained within one experiment were compared.

For most of our quantitative studies we used thick algal suspensions (i.e. in which practically all incident light of a wavelength $< 700 \text{ m}\mu$ was absorbed). In such suspensions the increase in dry weight is largely independent of the cellular concentration. Moreover, data obtained in this way, have general significance and bear directly upon the conditions of outdoor algal cultures (for which also thick suspensions are used). These experiments were carried out as follows:

In a thermostated bath a series of 6 identical 150 ml Erlenmeyer flasks could be exposed to white light of accurately known intensities. The vessels were illuminated with 24V, 150 Watt incandescent lamps with internal reflectors (Philips Attralux), mounted underneath the bath. These lamps each yielded upto $0.7 \text{ cal/cm}^2 \text{ min.}$ of photosynthizable radiation over an area of about 30 cm^2 . A sufficient amount of the algae to be studied was first grown in a larger vessel, exposed in one of the tanks. Each flask then was filled with 30 ml of a thick suspension of algae and continuously shaken and aerated with air containing 3 % CO_2 . Samples for dry weight estimations were taken at definite time intervals.

TEMPERATURE CHARACTERISTICS OF VARIOUS STRAINS

A number of strains, obtained as pure cultures from several sides¹ and kept on agar slants in a light cabinet, were tested in the described culture tanks and compared with strains, collected in Holland and used in earlier work.

The first screening consisted of a subjective judgement of the growth in the test tubes. Several strains grew in neither of the

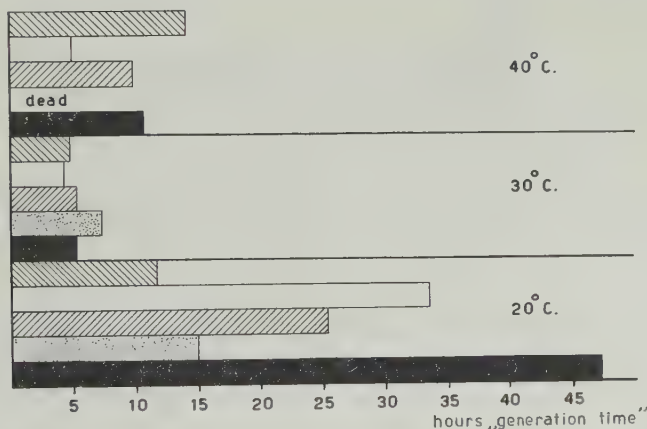


Fig. 1. "Generation-time" (see text) of a few strains of algae, grown in thin suspensions at a light intensity of $0.25 \text{ cal./cm}^2 \text{ min.}$, and temperatures as indicated, during the light periods: *Chlorella vulgaris* (strain Cornell), *Chlorella vulgaris* var. *viridis* (Yale) wild type, *Chlorella pyrenoidosa* (Emerson's strain), *Chlorella* Tx 1105, *Scenedesmus* 3.

¹ We wish to most gratefully acknowledge Drs APPLEMAN, DAVIS, MEFFERT, MYERS, PRINGSHEIM and WASSINK, for putting these strains at our disposal.

conditions; these were generally characterized by slow growth on the agar slants in the light cabinet as well, and were not used in further work.

An illustration of the quantitative differences in growthrate as observed in the three tanks, is given in fig. 1. For this experiment the test tubes were inoculated with algae obtained directly from slants in the light cabinet. (Similar results were found in case the algae had been pre-cultivated during a few days in liquid cultures at low light intensity and medium temperature).

For most strains, the optimal growth rate is found in the 30° C. tank. High temperature strain Tx 1105 grew about as fast at 40° C. as it did at 30° C. and in other experiments the shortest generation time was found at 40° C. *Chlorella vulgaris* var. *viridis* appears to be a low temperature strain, it grew relatively fast at 20° C., slower than the other strains at 30° C. and survived in no case at 40° C.

A peculiarity encountered in this type of experimentation was that the temperature characteristics of algal growth proved to be largely dependent upon the temperature of pretreatment.

The expt. given in fig. 2 was made with algae, which originated from the same agar slant, but had been cultivated at different

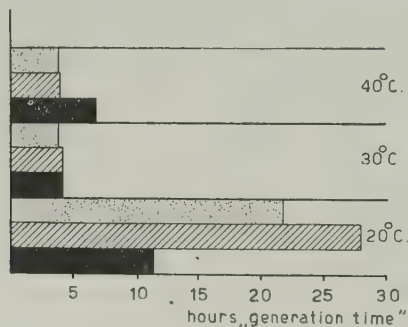


Fig. 2. *Scenedesmus* 3, cells cultivated during 7 months at: 20° C. (solid black), 30° C. (diagonal lines), 40° C. (white). Thin suspensions, exposed to a light intensity of 0.25 cal./cm² min., and the indicated temperatures. Expt. of November 1953.

temperatures during several months. In this case the algae which had been precultivated in the two warm tanks grew poorly at 20° C., and about equally fast at 30 and 40° C. The cells, pretreated at 20° C., gave similar results as the ones directly obtained from the light cabinet, and used for exp. fig. 1.

The same adaptation phenomena are illustrated in fig. 3, which shows the results of a growth experiment in which thick suspensions were used: Three samples of cells, adapted to 20, 30 and 40° C. respectively, were first exposed to 20° C., thereafter to 30° C., and finally to 40° C. It appears, that fastest growth occurred in case the temperatures of pretreatment and exposure were identical. The difference between 30 and 40° C. was—as usually—not very marked,

though. The growth rate of the cells, which were pretreated at 20° C., did not increase after the temperature was raised to 30° C. During the first day of exposure to 40° C. growth even ceased completely.

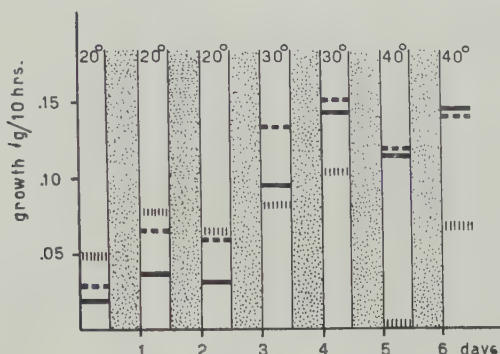


Fig. 3. *Chlorella*, 14-10, cultivated during 7 months at: 20° C. |||||, 30° C. ||||, and 40° C. ■■■. Intermittently exposed to 0.35 cal/cm² min. (10 hours light, 14 hours dark. Temperature during the light periods as indicated, temperature during darkness in all cases 20° C. Initial cell density: 0.1 g/30 ml. Expt. of November 4-14, 1953.

The algae used for expts. 2 and 3 had been preexposed to the three temperatures for over seven months. It was found, however, that for establishing (respectively reversing) the described temperature adaptations much shorter pretreatments sufficed. Usually, no further significant changes could be noticed after exposures during one or a few weeks.

From this we may conclude that a sharp characterisation of algal strains in terms of their relation between growth rate and temperature is not simple. But on the other hand, the *absolute* growth rate of "high temperature" strains, even after prolonged adaptation, was generally lower at 20° C. than that of "low temperature" strains and conversely, several "low temperature" strains did not survive at all in the warmest tank.

Strain *Scenedesmus* 3 appeared quite flexible in its temperature characteristics; at 20° C. it was the fastest growing strain tested and after short adaptation periods it grew about as well as any other alga at temperatures up to 45° C.

It can be noticed in figs. 1-3, that in all cases growth at 30° C. was much faster than at 20° C. At the lower temperature the cells showed a tendency for clumping and turning pale. The latter effect has to be ascribed to photo oxidation by the strong light in the thin suspensions. It is also noteworthy, that the growth rates observed in either thick or thin suspensions were hardly different at 30° and 40° C. and more or less equal for the (five) suitable strains tested so far at these temperatures.

It was also tried to grow alga at still higher temperatures (40 a 47° C. "day", 25° C. "night" temperature). Eventually after a short

adaptation, most strains which showed good growth in the 40° C. tank, thrived well up to 45–47° C., but not better than at 40° C. Further experiments and a more detailed discussion of these matters will be given in [2].

LIGHT CHARACTERISTICS OF THE ALGAE

The light sources used for irradiating the cultures in our tanks did not yield as high an intensity as prevailing under outside conditions in full sunlight. Still, this intensity was several times higher than that required for saturating photosynthesis in thin algal suspensions—even at the highest temperature used.

We tried to find out whether by prolonged cultivation at 0.25 cal/cm² min. the light saturation of growth could be adapted in a similar way as the temperature characteristics of the algae. No significant changes of the light saturation level were found, however, as is illustrated in fig. 4.

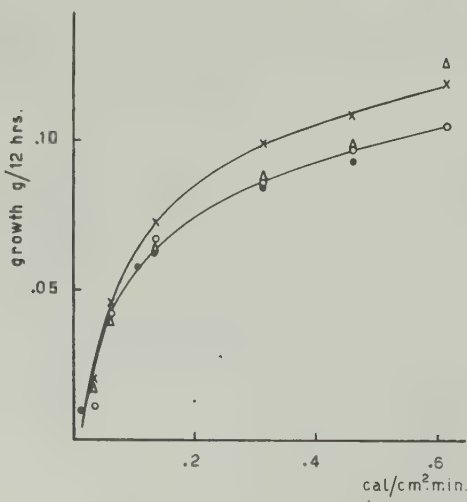


Fig. 4. Effect of prolonged exposures to 0.25 cal/cm² min. at 30° C. on the light saturation level of growth. Open circles: *Scenedesmus* 3, after one day exposure, closed circles: the same strain after 115 days exposure. Crosses: *Chlorella* 14-10 after one day exposure, triangles: the same strain after 115 days exposure. Cultures used for the short exposures were inoculated from agar slants and grown in weak light for a few days. Initial cell density: 0.08 g/30 ml. Temp.: 30° C. Expts. of August 7, 8, 11 and 13, 1953.

The light intensity available for our growth rate measurements in thick suspensions (0.6 cal/cm² min.) approached that of natural sunlight, so that it was of special interest to determine the yield of energy conversion attainable with various strains at different temperatures.

The amount of totally absorbed visible radiation ($\lambda < 700 \text{ m}\mu$) falling upon the vessels was determined by thermopile measurements

with and without an RG 8 filter (Schott & Gen.). The increase in dry weight of the algae was expressed in calories by taking one gram of dry algal material equivalent to 5.8 kcals. [3] The percent efficiency then was calculated as:

$$\frac{\text{cals. harvested material}}{\text{cals. absorbed radiation}} \times 100$$

At temperatures over 30° C. the better strains showed quite high efficiency values. At the highest intensity used (0.6 cal cm²/sec.) 8–10 % of the absorbed light energy was converted into cell material. At 20° C. generally much lower values (4–5 %) were observed.

OUTDOOR EXPERIMENTS

In order to compare simultaneously a number of different strains of algae, small culture flasks were used. For most exposures we used so called "Kolle" dishes (cf. fig. 5). Their flat shape facilitated an estimation of the irradiated surface (140 cm²) which was calculated as described in the legend of fig. 5.

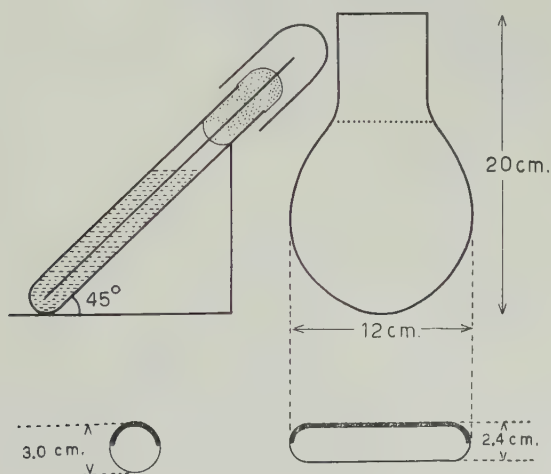


Fig. 5. To the right: a top view and a cross section of a Kolle dish. Top left: the position, in which the dishes were placed upon a blackened wooden support. Bottom left: cross section through a cylindrical vessel used. Its irradiated surface was computed by multiplying half of the circumference (heavy line) by the height of the fluid column. In the same way, for computing the exposed area of the Kolle dishes, half the circumference of the round sides was added to the area of the flat middle part.

Besides this, we used cylindrical tubes: with a radius (r) of 1.5 cm and a height of the fluid column (h) of 12 cm; it appeared, that the irradiated area of these tubes was best computed as $2 \pi r h$ (cf. fig. 5 and GEOGHEGAN [1] pag. 185). Calculated on this basis, the dishes and the tubes gave results fitting within $\sim 10\%$. A further check on these estimations was obtained by growing parallel samples of

algae in large flat vessels, for which commercially available "thermopane" windows ($40 \times 40 \times 1.2$ cm) were used. The vessels were exposed at an angle of 45° , facing the south and the suspensions were intensely flushed with air, enriched with carbondioxide. This arrangement made complicated stirring devices superfluous. The gas inlet tubes passed through cotton plugs, over which a glass cap served as a protection against rain.

The light intensity falling upon the cultures was recorded continuously. For this, a thermopile, enclosed in a glass hemisphere and mounted with the same tilt of 45° C. as the culture flasks, was used. The pile was connected to a galvanometer, which actuated a pen

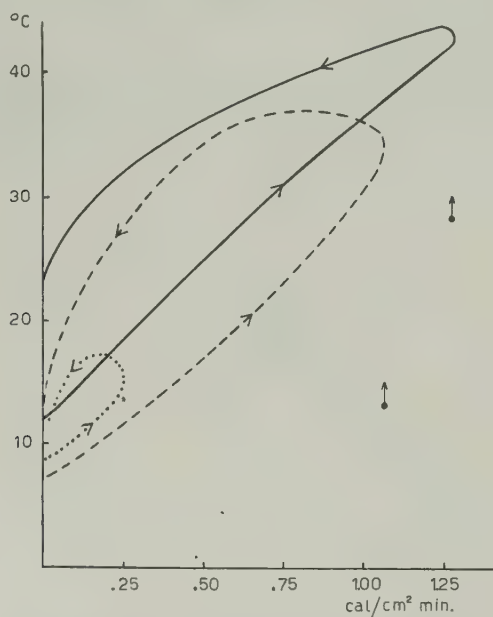


Fig. 6. Daily course of the temperature of the cultures in dependence of the light intensity. Solid line: July 25, broken line: Sept. 26, both cloudless days. Dotted line: Oct. 3, totally covered sky. The temperature of the surrounding air (dots) was measured at 1 p.m.

recorder via a photoelectric amplifier. From the area under the curves (cf. fig. 7) the sumtotal of light falling into the cultures during a given length of time could be calculated. The temperature was recorded with the aid of a simple thermograph, the feeler of which dipped into a culture tube filled with indian ink and exposed in the same way as the algal cultures. In addition, the air temperature was read twice daily.

It appeared that the sunlight falling into the vessels completely absorbed by the water and the algae, could raise the temperature considerably above the temperature of the surrounding air. On sunny days, differences over 20° C. were often observed in the middle of

the day, whereas on cloudy days, an effect was hardly present. This is illustrated in fig. 6, showing the correlation between light and temperature during the course of a few days, in which no abrupt changes in intensity occurred. In fig. 7 light- and temperature records are shown for two days, with varying cloudiness. In all curves the temperature appears to follow the light intensity with a rather small time lag. In a laboratory experiment, the temperature of a black

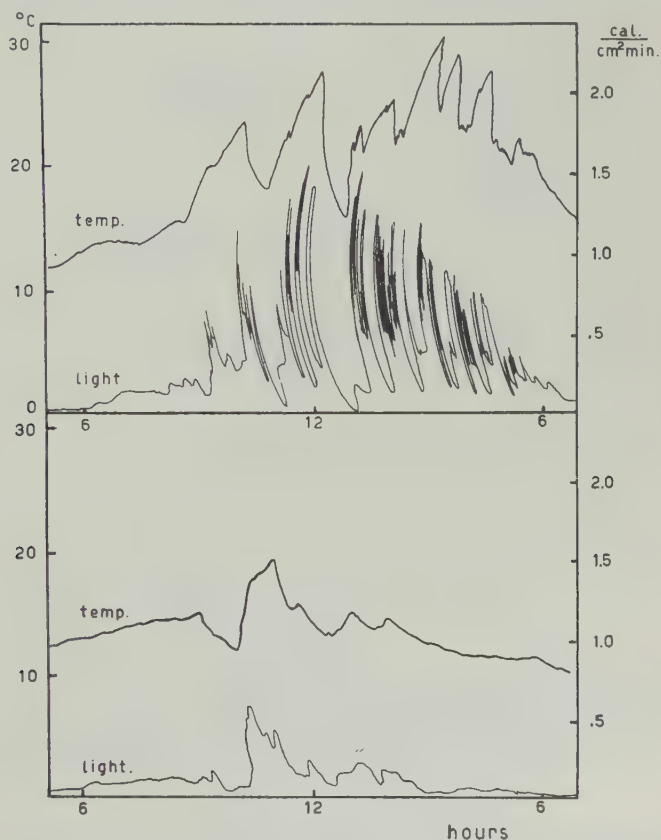


Fig. 7. Recordings of light and temperature on two days, both characterized by varying cloudiness. Top: July 30, bottom: July 31. Total radiation during these days: 199 and 66 cal/cm² min. respectively. In the upper figure short term temperature variations as large as 15° C. can be noticed.

liquid in a Kolle dish, if exposed to strong light, increased with a half time of about 25 minutes. Probably this parallelism between light and temperature has favourably influenced algal growth in our experiments.

If growth of the cultures was sufficiently fast, samples for dry weight estimation were taken daily. If growth was slower, this was done every other day, or at still longer intervals. At the moment a

sample was taken, exactly half of the culture was discarded and replaced by fresh, sterilized medium. By this procedure, the density of the cultures was always kept sufficiently high to ensure complete light absorption, so that independency of the growth rate of the cellular concentration was obtained.

From the increase in dry weight (expressed in grams per m² irradiated area), the efficiency of light utilisation could be calculated

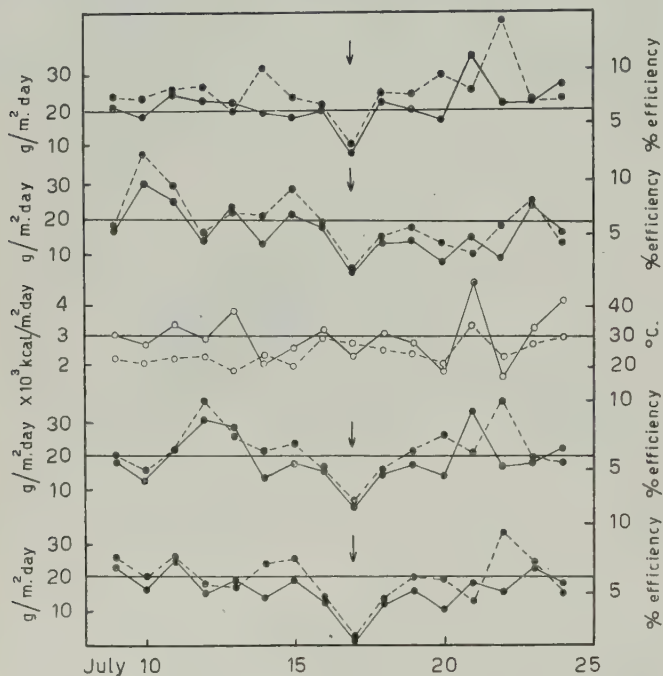


Fig. 8. Daily records from July 9-24, 1953, see text. Middle row (open circles): total radiation (solid lines) and average temperature (broken lines). In the other curves solid lines indicate daily yields, broken lines indicate efficiencies. From top to bottom: *Scenedesmus* 3, *Chlorella vulgaris* var. *viridis*, mixed culture Mt, mixed culture M 5-6-A. At ↓ the CO₂ supply failed.

for each period between two harvests. This calculation was made as described on page 539. We assumed that half of the amount of light measured by the thermopile was of a wavelength between 400 and 700 μ and therefore usable for algal growth, which is a rather conservative estimation.

In fig. 8 the daily amounts of total incident light, the average temperature and the growth of 4 algal cultures are plotted as observed during a few weeks.

Horizontal lines are arbitrarily drawn at a yield value of 20 grams/m² day. On the average the daily yields of *Scenedesmus* 3 (top row) surpassed this value. The fluctuations of these yields appear to be roughly correlated with the variation in the amounts of incident

light. The efficiency is found to be rather constant ($\sim 8\%$), except for a few high values (upto 15%), observed on days characterized by low radiation values. *Chlorella vulgaris* var. *viridis* (second row), a strain, which in the laboratory grew best at lower temperature (cf. fig. 1), indeed showed good yields during the first week characterized by low average temperatures. Yields decreased when the average temperature increased. Culture Mt (fourth row) contained

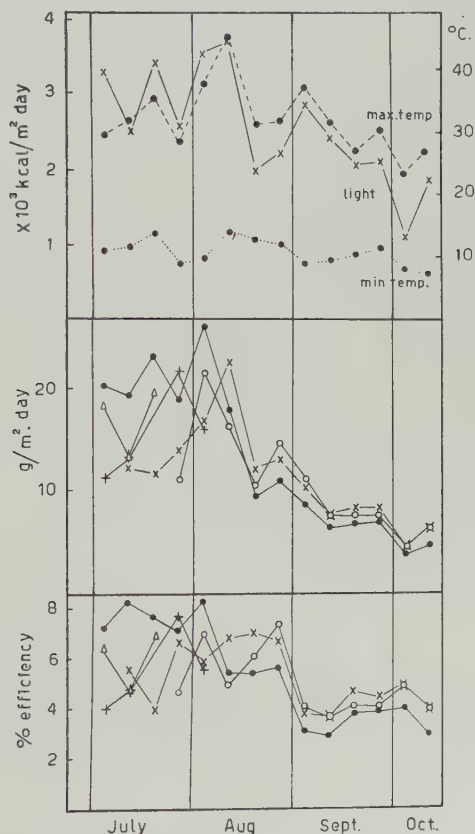


Fig. 9. Weekly averages from July to October 1954. Top section: solid line: incident light, broken line: maximum temperature, dotted line: minimum temperature. Middle section: yields observed for a few strains. Bottom section: efficiency of light utilisation as calculated from the above data. Cultures were initially inoculated with: ● *Scenedesmus* 3, ○ *Chlorella* Tx 1105, × *Chlorella* vulg. var. *vir.*, +: mixed culture 5-6-B, Δ: mixed culture 5-6-C.

a number of strains preselected at different temperatures in the thermostats, its growth curve resembles that of *Scenedesmus* 3 and follows the light curve still closer. Culture M 5-6 A (bottom row) contained a number of strains, preselected at 20°C . and showed lower yields on the average.

The growth of quite a number of strains was compared in exposures,

which generally did not last longer than a few weeks, since cross contamination of the cultures could not be excluded definitely.

Algae, characterized by good growth and high yields, were e.g. *Scenedesmus* 3, *Chlorella* Tx 1105 and 14-10 (MYERS strains [4]), *Chlorella* 7, *Chlorella* 19 and a few strains we newly isolated from tropical material.¹ Again the differences between "good" strains were only slight. The extreme variability of Dutch weather did not allow too detailed studies under outside conditions.

Fig. 9 shows weekly averages of the same type of data as given in fig. 8. Instead of the mean, the daily maximum and minimum

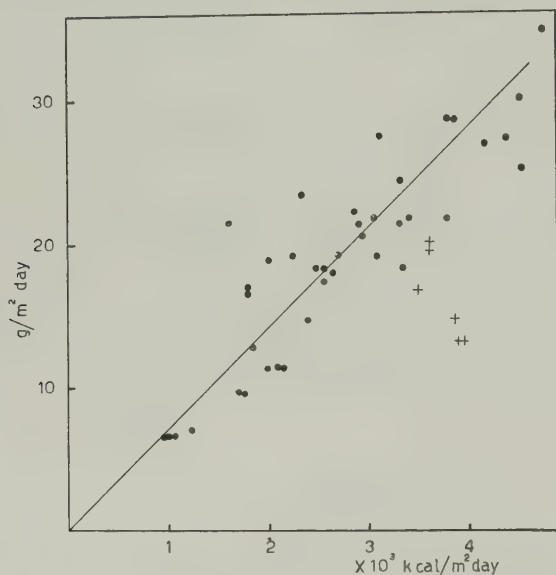


Fig. 10. Daily yields of *Scenedesmus* 3 during July and August 1953, plotted against the corresponding amounts of total radiation. Crosses represent harvests during about a week, during the first two days of which the maximum temperature of the culture surpassed 50° C. Growth rate was slowly resumed after this. The straight line indicates an efficiency of 8 %.

temperatures are given in this figure. The maximum temperature appears even more closely correlated with the light intensity, as was the mean temperature (cf. fig. 8). The minimum temperature was rather constant and low during the whole period and the often quite large differences with the maximum temperature are noteworthy.

For 3 cultures the yields and efficiencies were observed during the whole growing period. The yields of two mixed cultures, exposed 6 weeks, are plotted in addition. As also fig. 8 already showed, the mixed cultures did not prove to be superior to monocultures and were not used further.

Strains with favourable light and temperature characteristics were selected from crude material kindly collected by Dr F. K. VAAS in

The curves of fig. 9 indicate high yields (up to 25 g/m² day) and efficiencies in the order of 6–8 % during July and the first part of August. The highest yields were observed in the first week of August, characterized by high radiation and high temperature. At the end of the second week of August, however, the maximum temperature in most cultures surpassed 50° C., which led to a decrease of the yields and bleaching of the cells. Growth was resumed only slowly. Unfortunately the solar radiation remained on a low level after this recovery period and yields as high as before were not recorded again during the latter part of the experimentation period. During September and October, a much lower and about constant efficiency of about 4 % was found. This general trend to decline during the later part of the growing season is not only shown by the yields but by the growth efficiency as well and is quite remarkable. It was found in earlier work in much the same way and will be further discussed by one of us [2]. Still, the observed yields and efficiencies at all times were considerably higher than the ones we observed earlier [1] p. 55. In fig. 10 the daily yields observed during July and August for the culture of *Scenedesmus* 3 are plotted against the total radiation. The straight line represents an efficiency value of 8 %, and serves as an index only.

The more or less linear relationship between daily yield and quantity of light up to high values of both is striking. It may be due, at least partly, to the correlation of light intensity and temperature in the vessels. We hope that further experiments now under way will contribute to a more complete understanding of the complicated relationships between the effects of light intensity, temperature and suspension density under the conditions of these experiments.

ACKNOWLEDGEMENT

We want to express our appreciation to Miss C. Janse and Mr. A. J. Luitingh for diligent technical assistance throughout this work.

SUMMARY

Various strains of algae were tested under conditions of high light intensities at different temperatures. Differences in growth rate were studied and the better strains selected. By prolonged exposures it was tried to further adapt promising strains to such conditions. No increase of the light saturation level of growth could be noticed after seven months exposure to intensities of 0.25 cal/cm² min. On the other hand, for several strains an exposure during some weeks appeared to suffice for adapting the cells to a given temperature.

During the summer of 1953 growth yields of various promising strains were studied in small outdoor culture devices. Average yields of 20 g/m² day and efficiencies of light energy conversion of 8 % were observed during July and August. Later in the season both yields and efficiencies declined. These figures imply that during the

whole growing period the observed yields were considerably (about twofold) higher as those found in our earlier algal culture experiments.

LITERATURE

- [1] BURLEW, J. S. (Ed.) 1953. Algal culture: From laboratory to pilot plant, Carnegie Institution of Washington Pub. 600.
- [2] OORSCHOT, J. L. P. VAN, thesis, in preparation.
- [3] KOK, B. 1952. *Acta Bot. Neerl.* 1: 445-467.
- [4] SOROKIN, C. and J. MYERS. 1953. *Science* 117, 338.

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